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Merging Medicine with Science

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It is with great pleasure that we present the Proceedings of the NHG Annual Scientific Congress 2004 as a supplement issue in the *Annals of the Academy of Medicine, Singapore*. For the third year running, the NHG member institutions have come together for the Annual Scientific Congress (ASC). Through the ASC, we hope to promote research and foster close relationship and camaraderie among health professionals under the large NHG umbrella. It is through this Congress that we aim to showcase our research and bring important advances in Medicine and Science to all our NHG staff.

Last year for NHG ASC 2003, we saw probably the largest number of abstracts ever submitted to a local medical meeting; 481 abstracts were submitted and 392 were accepted after peer review. Although the standards were generally good, we wanted to reduce the number of rejected abstracts (18.5%) by helping our staff to plan, carry out and present their research more effectively.

To achieve our aims, we held a series of 3 workshops to generate increased interest in research and maximise their quality. These included workshops on “How to Plan & Formulate a Research Project”, “How to Write an Abstract/Extended Abstract”, and “How to Design a Scientific Poster & Prepare an Oral Presentation”. The workshops were sponsored by a generous grant from the NHG Endowment Fund. Each of these workshops was well attended by almost 200 participants.

We were delighted by the 471 abstracts submitted this year. After the same process of peer review, only 21 (4.5%) of abstracts were rejected, giving a whopping number of 450 abstracts accepted for publication in this supplement issue of *Annals*. Our reviewers were impressed by the improved quality of abstracts submitted.

To allow various types of research to be showcased in this Congress, we have organised the abstracts into special categories featuring various specialties, providing separate sessions for clinical and laboratory research, showcase Clinical Practice Improvement Programmes, and dedicated sessions for Nursing and Allied Health research. The prestigious NHG Doctor Award was created for the top abstracts submitted in the area of clinical research. The NHG Young Investigator's Award was dedicated to young researchers especially in the areas of laboratory-based research. We carefully selected the top 49 abstracts for extended abstract submission to better showcase their quality. Thirty of these abstracts were selected for oral presentation during the Congress. Due to the limited space available in the Congress venue, we have to divide the poster presentations over 2 days during the Congress.

The breadth of the abstracts submitted to this Congress has been breathtaking. We were greatly encouraged by the broad range of topics and research covered. Many of the research abstracts submitted showed the true spirit of Merging Medicine with Science, where questions arising from care of the patient is addressed by good scientific research. We were also delighted that every member institution of NHG had submitted abstracts to this Congress.

This supplement issue of the *Annals* will not be possible without the tremendous support of researchers and participants who have strived to put their good research on paper. We would like to thank our large panel of reviewers for their timely review and comments, which enabled us to put together this supplement issue. The efforts of our IT support by making it possible for presenters to submit abstracts and reviewers to judge these abstracts electronically have enabled us to meet the tight timeline. Last but not least, we thank the NHG College staff, especially Ms Cindy Chen, and the *Annals* editorial staff for their kind editorial support for this issue.

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Merging Medicine with Science: The Birth of a Targeted Therapy in Cancer

J Mendelsohn,¹MD

We will review the development of a new anticancer therapy which acts by blocking the EGF receptor (EGFR) protein on the surface of cancer cells. The research began in 1981 and has not ended. Participants have included scientists in universities, biotech and pharmaceutical companies, the US National Cancer Institute, the Food and Drug Administration (FDA), clinical investigators at many cancer centres, and thousands of patients.

EGFR are expressed at high levels in about 1/3 of epithelial cancers, and autocrine activation of EGFR appears to be critical for the growth of many tumours. In 1981 Dr. Gordon Sato and I hypothesised that blockade of the binding site for EGF and TGF- α on EGFR with an antireceptor monoclonal antibody (mAb) might be an effective anticancer therapy by inhibiting activation of the receptor tyrosine kinase. Murine mAb 225 inhibited EGFR tyrosine kinase, and inhibited tumour cell growth in cultures and in nude mouse xenografts. C225 is the human:mouse chimeric version of mAb 225, administered intravenously in clinical trials. Pharmaceutical companies have developed a number of oral, low molecular-weight inhibitors which act intracellularly on the ATP binding site of EGFR, also blocking receptor activation. These molecules differ in their specificity for the EGF receptor and their reversibility of binding. The mechanisms of tumour inhibition by these anti-EGF receptor agents involve growth inhibition through upregulation of p27^{Kip1}, enhancement of apoptosis, and inhibition of angiogenesis and metastasis. In addition, these agents enhance the cytotoxicity of chemotherapy and radiotherapy in experimental systems. In the case of radiation therapy, inhibition of EGFR function results in inhibition of radiation-induced DNA damage repair. In the case of mAb C225, immune mechanisms may contribute to the antitumour activity. The mechanisms which may contribute to the activity of these agents against cancer are summarised in Table 1.

Table 1. Acquired Capabilities of Cancer Cells (Hanahan and Weinberg, Cell, '00)

Characteristics	Increased by EGFR stimulation	Decreased by EGFR inhibition
Self-sufficiency in growth signals	yes	yes
Insensitivity to antigrowth signals	yes	yes
Evading apoptosis	yes	yes
Limitless replicative potential	yes	yes
Sustained angiogenesis	yes	yes
Tissue invasion and metastasis	yes	yes

These findings in extensive preclinical studies led to clinical trials of EGF receptor inhibitors, both as monotherapy and in combination with chemotherapy or radiotherapy. Results from Phase I and II trials involving thousands of patients are promising, and data from Phase III trials have appeared. In total, nearly a dozen different experimental molecules that act by inhibiting the EGF receptor are in the clinic. The reported results from Phase II trials, randomised trials, and trials of combination therapy have shown response rates in the range of 0% to 25%. The most common toxicity is an acneiform rash, which may identify potential responders to therapy. The oral agents, but not C225, cause diarrhoea as a dose-limiting toxicity. One oral agent, Iressa, has been approved by the FDA as monotherapy treatment for advanced, refractory non-small cell lung cancer. On February 12, 2004, the US FDA approved C225 (Cetuximab, Erbitux[®]) for treatment of advanced colorectal cancer.

Many challenges remain to be addressed in the clinical application of anti-EGF therapies. Is EGF receptor signalling different in cancer cells expressing 10⁶ receptors than in normal cells expressing 10⁴? Why do some but not all patients with an EGF receptor-expressing cancer respond to receptor inhibitors? Are there markers that could identify responsive cancers? What are the specific mechanisms for synergism between EGF receptor inhibitors and chemotherapeutic agents or radiation, and between EGF receptor inhibitors combined with agents promoting apoptosis or blocking angiogenesis? Do the differences between mAbs and low molecular-weight inhibitors of EGFR translate into differences in clinical activities? These questions suggest the need for further preclinical studies, for carefully targeted clinical trials that measure molecular effects of therapy, and for ways to speed up the sequence of trials required to evaluate new therapies that may work best in combinations.

Many lessons have been learned from the experience of discovering a cancer treatment and bringing it to the clinics, a process that in this case took 22 years. Some of these include:

- When new therapies targeting genetic or molecular abnormalities in cancer cells are discovered, research-seeking markers to identify patients likely to respond should be initiated early, in parallel with late preclinical studies and initial clinical trials.
- Phase I trials must be designed to establish the optimal biological dose in addition to the maximum tolerated dose – necessitating well designed and adequately funded pharmacodynamic studies of tumour tissue with molecular assays and PET imaging.
- Agents targeting a single gene or protein are not likely to produce high response rates in cancers which have multiple genetic abnormalities, so combination therapy must be encouraged early in the approvals process.
- Universities must place greater emphasis on nurturing, incentivising and promoting faculty with strong skills and long experience in clinical trials research. They require protected time for planning and carrying out experiments, similar to laboratory researchers.
- The off-label use of anticancer agents alone and in combinations, in the setting of vetted clinical trials, should be encouraged and funded.
- Improved clinical trial design can move through the sequence of Phase I-II-III clinical trials more rapidly by building on previous data.
- FDA regulations should encourage innovation in the drug approval process while protecting the public. This will require more flexibility and openness to expert consultation early in the process of clinical trial design and approval.

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Bone Marrow Transplantation for Leukaemia – Current Status and Strategies for Improvement[†]

FR Appelbaum,¹MD

Introduction

Over the last 35 years, bone marrow transplantation (BMT) has evolved from a highly experimental therapy to a well-established treatment used in the management of tens of thousands of patients annually. The International Bone Marrow Transplant Registry (IBMTR) estimated that approximately 17,000 allogeneic and 30,000 autologous transplants were performed in 2003.¹ Despite its widespread use, marrow transplantation is unavailable to some patients because of the lack of an appropriate source of stem cells, and in many others the treatment fails because of excessive toxicity or an inability to eradicate the disease for which it is being used. The following discussion will briefly review the current status of BMT in the treatment of leukaemia and will discuss some strategies being pursued to improve the technique.

Current Status of Bone Marrow Transplantation for Leukaemia

Acute Myeloid Leukaemia (AML)

Allogeneic bone marrow transplantation is the only form of therapy able to cure patients who fail initial induction or re-induction therapy, with 15% to 20% of such patients becoming long-term disease-free survivors in each case.² Allogeneic transplantation cures 35% to 40% of patient transplanted in second complete remission, results that are substantially better than can be achieved with chemotherapy.³ If conducted in first remission, allogeneic transplantation results in cure rates of 45% to 65%. Whether or not these results are superior to that which can be achieved with either autologous transplantation in first remission, or a strategy of an initial trial of chemotherapy followed by transplantation as salvage treatment has been the subject of much discussion and a number of prospective trials. A large prospective trial (AML 8) by the European Organization of Research and Treatment of Cancer (EORTC) reported 4-year disease-free survival (DFS) of 55% with allogeneic transplantation, 48% with autologous transplantation and 30% with chemotherapy.⁴ A recent update of a similarly designed North American Intergroup study reported survival at 5 years of 52% with allogeneic transplantation, 42% with autologous transplantation, and 39% with chemotherapy.⁵ The EORTC performed a second study (AML 10) with the goal of comparing allogeneic to autologous transplantation for AML in first remission and, as in their first study, found an advantage with allogeneic transplantation (4-year DFS 51.4% versus 41.2%, $P = 0.04$).⁶ In contrast the Groupe Ouest Est Leucémies Aigues Myéloblastiques (GOELAM) trial, with a design similar to the EORTC AML 8 trial, saw no advantage to either form of transplantation as compared with chemotherapy.⁷ Finally, the Medical Research Council of the United Kingdom compared 3 cycles of intensive consolidation chemotherapy followed by autologous transplantation with consolidation chemotherapy alone and found fewer relapses and improved disease-free survival with the autograft, but no difference in overall survival.⁸ None of these

studies were prospectively designed to address the comparative utilities of these approaches for specific sub-groups of AML patients, but a recent retrospective study suggested a particular benefit of allogeneic transplantation for patients with high risk disease.⁵

Acute Lymphocytic Leukaemia (ALL)

As in AML, allogeneic transplantation is the only curative therapy for adults with ALL who fail initial induction or re-induction therapy, curing 10% to 20% of such patients.² Five-year survival for adults treated with allogeneic transplantation in second remission averages around 30%, which is much better than expected with chemotherapy. There has been only 1 large prospective study so far published comparing chemotherapy with allogeneic or autologous transplantation for patients in first remission. That study found an advantage for allogeneic transplantation over the other 2 approaches (5-year DFS of 46% versus 31%), with most of the advantage in high-risk patients (DFS 44% versus 11%).⁹ Because the outcomes of chemotherapy for ALL are better in children than adults, the indications for transplantation are more limited. Nonetheless, children who relapse on therapy or within 6 months of its completion benefit from allogeneic transplantation. Children with high-risk disease in first remission, particularly those with Ph⁺ ALL, also appear to do better with transplantation compared to chemotherapy.¹⁰

Chronic Myeloid Leukaemia (CML)

Allogeneic or syngeneic transplantation is only known cure for CML with 5-year disease-free survival rates of 15% to 20% for patients transplanted in blast crisis, 20% to 50% for accelerated phase patients, and 50% to 75% for chronic phase patients.¹¹ For chronic phase patients, the interval from diagnosis to transplantation influences the outcome of the procedure with the best results seen in patients transplanted within one year of diagnosis, and progressively poorer results with increasing delay. Results using unrelated donors now approach those with matched siblings, given recent advances in donor selection, graft-versus-host disease (GVHD) prophylaxis and supportive care.¹² Although pilot studies of autologous transplantation for CML show that temporary conversion to Ph negativity is possible, there is no proven role for autologous transplantation in the management of CML.

With the availability of imatinib, the role of allogeneic transplantation in the treatment of CML has become more complex. Because very few patients achieve a molecular complete remission with imatinib and therefore are not cured, many would argue that allogeneic transplantation remains the treatment of choice for younger patients with matched siblings. For older patients and those without matched siblings, an initial trial with imatinib is reasonable to see if a complete cytogenetic response can be obtained. For those who do not obtain a complete cytogenetic response and those who show regrowth of the malignant clone after achieving a response, transplantation should be considered without undue delay.

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Chronic Lymphoid Leukaemia (CLL)

Because of the indolent nature of CLL and the relatively advanced age of most patients, the experience with bone marrow transplantation as treatment for this disease is relatively limited. Our experience in Seattle using allogeneic transplantation to treat patients who have failed on average 4 different prior therapies showed a 56% 5-year disease-free survival, not too different from the 49% survival reported by the IBMTR and the 54% 3-year disease-free survival reported by the European Bone Marrow Transplant Registry.¹³ Experience with autologous transplantation in CLL is very limited.

Strategies to Improve the Outcome of Transplantation for Leukaemia

Improved Tumour Ablation

Although the high-dose preparative regimens commonly used in transplantation, such as busulfan plus cyclophosphamide, or cyclophosphamide plus TBI, are sometimes effective in curing patients, too often patients die from toxicities induced by such regimens or their disease recurs despite the high dose of therapy administered. A number of approaches are being taken to improve this situation.

Pharmacologic approaches: One set of studies found considerable variability in the plasma busulfan concentrations seen following administration of a busulfan-cyclophosphamide conditioning regimen. Of particular interest was the observation that the majority of relapses occurred in patients with busulfan plasma concentrations below the median while very high plasma concentrations were associated with excess toxicity.¹⁴ By targeting busulfan levels based on the metabolism of the initial dose, both excessive toxicity and undertreatment with increased relapse rates could be avoided. We have since found similar variability in the metabolism of cyclophosphamide and have reported a tight correlation between high levels of a specific cyclophosphamide metabolite, carboxylethyl phosphoramidate mustard, and non-relapse mortality.¹⁵ We are now testing whether adjusting cyclophosphamide dosing based on the metabolism of the initial dose can prevent excess toxicity following marrow transplantation.

Targeted radiotherapy: Prior randomised trials comparing different doses of TBI have reported decreased relapse rates with higher doses of TBI, but increased non-relapse mortality nullifying any overall benefit from the more dose-intensive regimens.¹⁶ In an effort to capture the benefit of increased dose without increased toxicity, we and others have explored the use of monoclonal antibodies to target locally acting radionuclide to sites of disease, thereby increasing the dose of radiation to tumour, while sparing normal organs. Encouraging results of phase II studies using an anti-CD20 radiolabelled antibody in combination with cyclophosphamide and etoposide as the preparative regimen for patients with B-cell lymphoma have been published, as have data using a preparative regimen of a radiolabelled anti-CD45 antibody combined with busulfan plus cyclophosphamide for patients with AML.^{17,18}

Specific Immunosuppression

Preparative regimens used in allogeneic transplantation for haematologic malignancies have typically been composed of high doses of relatively non-specific reagents, such as TBI and alkylating agents, in part, because of presumed contributions of such treatment to the eradication of the patients' malignancies and, in part, because of the belief that such high-dose therapy was required to ensure engraftment. It has, however, long been appreciated that much of the anti-leukaemia effect of allogeneic transplantation derives from a graft-versus-tumour (GVT) effect, a view that has been strengthened

by the ability of donor lymphocyte infusions to induce remissions in some patients who have relapsed post-transplant. In an effort to capture the benefits of the GVT effect in patients too old or infirm to tolerate high-dose therapy, a number of reduced-intensity preparative regimens have been developed. A very low-dose regimen employing fludarabine 30mg/m² for 3 days plus 200 cGy TBI with post-transplant mycophenolate mofetil and cyclosporine was developed based on pre-clinical studies. Application of this regimen to a substantial number of patients, including many in their sixth or seventh decade, shows that complete engraftment of match sibling or match unrelated stem cells can be achieved in virtually every case with overall non-relapse mortality rates of less than 10% at 100 days and less than 20% overall.¹⁹ Complete responses have been documented in patients with a variety of hematological malignancies. The most encouraging results to date have been in patients with less tumour burden and those with more indolent malignancies, such as chronic leukaemia, and follicular lymphoma. Combining targeted radiotherapy, with a non-ablative allogeneic transplantation is an attractive approach currently under study.

Optimising the Stem Cell Source

Allele-level typing of unrelated donors: Only approximately 25% of individuals have HLA-identical siblings to serve as donors, and therefore many patients must rely on unrelated donors. In most studies to date, both GVHD and graft rejection have been more common following matched unrelated transplants than with matched siblings. Part of the explanation is that in the past HLA typing relied on serologic methods that did not identify all HLA subtypes. A recent study using automated direct sequencing of HLA-A, -B, -C, DRB1 and DQB1 found allele level mismatching in 30% of donor-recipient pairs previously thought to be HLA matched.²⁰ This study also showed that such allele level mismatching resulted in more GVHD and graft-rejection, a finding that argues that more careful selection of unrelated donors should improve transplant outcome.

Typing for non-HLA polymorphisms: A number of investigators have hypothesised that polymorphisms in cytokine genes might influence inflammatory and immune responses post transplant. In an analysis of 993 transplants, those recipients with an IL10 promoter genotype AA had a significantly reduced risk of GVHD ($P = 0.02$) and reduced non-relapse mortality compared to other genotypes.²¹ This finding suggests that there may be additional polymorphisms affecting outcome that might influence donor selection and patient prognosis.

Alternative stem cell sources: Although matched sibling or unrelated donors can be found for approximately 70% of patients, for others, including a disproportionate number of African-Americans and Hispanics, other sources of stem cells are needed. Umbilical cord blood banks provide an alternative for many children, but results of such transplants in adults have been less encouraging due to the low cell content of most cord blood collections. The use of multiple cord blood units for transplantation shows considerable promise for overcoming this limitation.²² Progress has also been made in the use of haplo-mismatched donors as well.²³ One technique combines a high CD34 cell dose with vigorous T-cell depletion, and a second uses high-dose post-transplant cyclophosphamide to eradicate alloreactive T-cells.

Harnessing the Power of the Graft-versus-tumour Effect

As noted earlier, the observation of markedly diminished relapse rates associated with GVHD and the responses seen with donor lymphocyte infusions have generated enormous interest in developing ways of harnessing the GVT effect while avoiding the toxicities of

GVHD. One approach is to transduce donor T-cells with a “suicide” gene, allow GVHD to develop and then rescue patients by triggering the “suicide” gene and thereby eliminating GVHD.²⁴ Other approaches are centred on identifying antigen targets that distinguish GVT from GVHD. One category of targets is polymorphic minor histocompatibility antigens that are restricted to hematopoietic tissue and differ between donor and host. A number of such antigens have been identified and are being exploited as targets for T-cell adoptive immunotherapy post-transplant.²⁵ A second category of antigens are those associated with the malignant phenotype such as mutational antigens (e.g. bcr/abl in CML) or overexpressed self antigens (e.g. PR3 in AML).

Summary

Thirty-five years ago, bone marrow transplantation was first being explored as a last-ditch effort to treat patients with end stage leukaemia. Through the efforts of a large number of laboratory and clinical scientists, the application of transplantation has broadened and outcomes have dramatically improved. The science of transplantation continues to attract a great deal of research, and with this effort we can expect continued progress and patient benefit.

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Stem Cell and Cartilage in Orthopaedics

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In the past decade, much has been learnt about the regeneration and repair of skeletal tissues. Regeneration involves slow replacement of tissues with identical tissue. It occurs readily in the embryo, hardly at all in neonates, and is never observed in adults. In 1743 Hunter¹ stated, "From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that, once destroyed, is not repaired." A now extensive knowledge base of both repair and regeneration in orthopaedics tissues has enabled the development of new and innovative treatment modalities.

Articular cartilage is unique avascular, aneural and alymphatic load-bearing live tissue which is supported by the underlying subchondral bone plate. It is unique in that the extra cellular matrix is composed of a complex combination of type II collagen fibrils which are specifically arranged and have bonded to them very large water-retaining molecules called aggrecan molecules. This combination of molecules gives articular cartilage its unique ability to resist the repetitive compressive load-bearing necessary for the activities of daily life without undergoing premature repair.

Articular cartilage injuries have a limited potential to heal, which, over time, may lead to osteoarthritis.^{2,3} Cartilage defects in the knee may cause pain, swelling and catching. There are several different surgical procedures available to treat cartilage injuries, but no method has been judged superior. The ultimate aim of treatment is restoration of normal knee function by regenerating hyaline cartilage in the defect and complete integration of the regenerated cartilage with the surrounding cartilage and underlying bone. Chondrocytes are responsible for the unique features of articular cartilage; it seems rational to use truly committed chondrocytes to repair an articular cartilage defect.

Autologous chondrocytes cells expanded in vitro and combined with periosteum were first implanted in articular cartilage defects of patients in 1978.⁴ This first generation of chondrocytes transplantation procedures was initially termed autologous chondrocytes transplantation. Today, the technique is called autologous chondrocytes transplantation or autologous chondrocytes implantation (ACI). Implantation consists of an arthrotomy, preparation of the defect, (Fig. 1) harvest of a periosteal flap, fixation of the periosteal flap to the defect, (Fig. 2) securing a watertight seal with fibrin glue, implanting the chondrocytes, (Fig. 3) and wound closure.

ACI resulted in the formation of new cartilage that was similar to normal cartilage in that it had an abundance of type II collagen and metachromatically stained matrix. The chondrogenic cells in the transplant may be able to repair cartilage more efficiently than the chondrocytes at the margin of the injured cartilage. The culturing procedure increased the number of chondrocytes initially isolated by 10 to 20 times. A fraction of the cultured cells were able to re-express their chondrogenic phenotype, after the use of culturing procedures known to facilitate the production of cartilage matrix.⁴ The tissue that results from reparative techniques such as drilling and abrasion is disorganised fibro cartilaginous tissue with type I collagen fibres^{5,6}

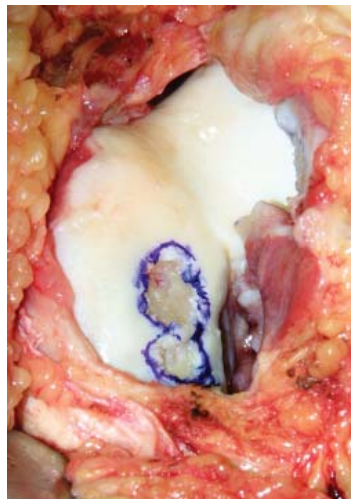


Fig. 1. Intraoperative photograph of a left medial femoral condyle chondral ulcer in 43-year-old female.



Fig. 2. Intraoperative photograph demonstrating a periosteal flap stitched over patella ulcer.

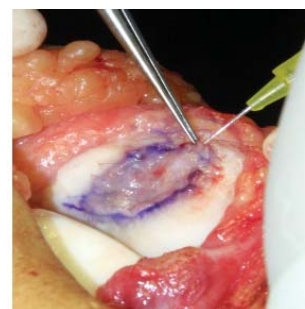


Fig. 3. Intraoperative photograph demonstrating a chondrocytes injected under periosteal flap on patella.

that is unable to restore the biomechanical properties of normal articular cartilage.⁷

Initially, ACI was limited to relatively small or medium-sized focal chondral and osteochondral defects of the weight-bearing surfaces of the femoral condyles and the patellofemoral joint. As notable success was obtained in these joints, the indications were extended to other diarthrodial surfaces, including talar, tibial, humeral capitular, and recently, femoral head lesions. Theoretical and practical considerations suggest that the ideal diameter of the defect is between 1 and 4 cm². Usually, both of the patellofemoral peripheries allow graft harvest for defects of 3 to 4 cm². Under certain conditions the ACI can be used as a salvage procedure for defects as large as 8 to 9 cm², but such extension of the indication can result in a higher rate of donor-site morbidity.⁸

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In treating patients, it is important that ACI is recognised as only 1 element. In every case, it is necessary to treat any additional joint abnormalities that would contribute to the failure of the joint. Accordingly, the treatment of instability, malalignment and meniscal and ligament tears must be incorporated in the operative and postoperative rehabilitation algorithms. ACI permits an immediate full range of motion, but requires 2 weeks of non-weight-bearing and an additional 2 to 3 weeks of partial weight-bearing after the operation.

However, there are some concerns about the procedure as it is currently performed. For example, it is 2-step procedure (with 1 step being an open arthrotomy), and the technique of suturing the periosteum is tedious and cumbersome. In addition it is expensive and somewhat technique-dependant.⁹ The question of chondrocytes phenotype and stability has not been effectively quantitated with use of this technique. Nonetheless, it has changed our view of the biological potential for the repair of articular cartilage. The success of this procedure has spawned a number of new protocols involving various cell-based therapy systems to elicit cartilage repair.

Many adult tissues contain populations of stem cells that have the capacity for renewing themselves after trauma, disease or aging. The cells may be found within the tissues or in other tissues that serve as stem cell reservoirs. For example, bone marrow not only is the major source of adult hematopoietic stem cells (HSCs) that renew circulating blood cells, but also contains mesenchymal stem cells, now referred as bone marrow stromal stem cells (MSCs), which contribute to the regeneration of mesenchymal tissues such as bone, cartilage, fat, tendon, muscle and stroma. MSCs can be isolated from other cells in bone marrow by their tendency to adhere to tissue culture flasks and density gradient centrifugation, which presents an intriguing model for examining the differentiation of stem cells.

Presently researchers focused on the use of periosteal-derived stem cells for repair of osteochondral defects for a number of reasons. The first reason is that these cells are easily expanded in culture and can be phenotypically stable. Also, they are ideal for the delivery of various genes promoting the repair, maintenance, and anabolic

metabolism of cartilage in lesions. Candidate genes for this application include bone morphogenic protein-7 (BMP-7), insulin-like growth factor-1 (IGF-1), and possibly transforming growth factor-beta (TGF-beta).⁹

Today tissue engineering is a field of biomedicine that is grows rapidly, and cell biologists, engineers and surgeons have to work closely together to reduce the gap between where the cartilage repair technology is today and where we want it to be. This interdisciplinary repair technology may be named biomedical surgery, and its novel instruments include embryonic stem cells, pluripotent mesenchymal stem cells, morphogens, smart biomaterials and gene transfers, which the different researchers should try to make into a biological well-tuned orchestra.

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Endocrinological Aberrations in Survivors of the Severe Acute Respiratory Syndrome (SARS) in Singapore

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Introduction

Ever since Singapore was stricken in March 2003 with severe acute respiratory syndrome (SARS), it became apparent that many survivors experienced chronic morbidities including a multitude of psychosomatic manifestations.

We hypothesise that these symptoms could have an endocrinological basis due to their resemblance to various hormonal and metabolic disorders that frequently feature non-specific symptomatology.¹ Studies have linked aberrations in the hypothalamic-pituitary-adrenal (HPA) axis to psychiatric conditions.² Moreover, correlations of thyroid dysfunction with certain psychosomatic syndromes have been described.³

The primary focus of this prospective study is to determine the existence of any chronic endocrine sequelae in SARS survivors, while characterisation of their prognostic outcomes constitutes its secondary objective. Given that the endocrinopathic properties of the SARS-associated coronavirus (SARS CoV) is currently an inadequately explored domain, any hormonal aberrations unravelled through this preliminary investigation will contribute new scientific insights to the medical database with the potential of translating into clinically relevant therapeutic strategies for SARS-associated endocrinopathies and paradigm shifts in treatment approaches to endocrine disorders as future spin-offs.

Materials and Methods

Subject Recruitment

This research was conducted in Tan Tock Seng Hospital with approval from its Institutional Review Board. All survivors of probable SARS in Singapore aged 21 years and above were eligible. Sixty-one provided written informed consent and were prospectively enrolled about 3 months post-discharge. Those with pre-existing endocrine disorders before SARS were excluded.

Sample Collection and Processing

Blood samples were collected at 0800 h by venepuncture into EDTA and plain tubes for: 1) full blood count, 2) electrolytes, 3) cortisol, 4) adrenocorticotrophic hormone (ACTH), 5) free thyroxine (FT4), 6) free triiodothyronine (FT3), 7) thyroid stimulating hormone (TSH), and 8) dehydroepiandrosterone sulphate (DHEAS). 24-hour urinary cortisol was done to assess integrated cortisol secretion. Hormone measurement techniques including immunochemiluminometric assay (ICMA) and radioimmunoassay (RIA) were employed according to standardized protocols. Subjects with serum cortisol below 275 nmol/L as cutoff underwent dynamic HPA axis evaluation using low dose (1 mcg) short Synacthen test (SST). Subjects with 8 am serum cortisol below 138 nmol/L and/or post-stimulation serum cortisol under 550 nmol/L at 30 minutes were deemed hypocortisolic. SST assessment for HPA axis recovery was repeated at 3 to 6 monthly intervals. Although the study was terminated a year following diagnosis of HPA axis dysfunction,

reviews were extended for those with persistent hypocortisolism as medically appropriate. Symptomatic patients with orthostatic hypotension were prescribed physiological doses of hydrocortisone replacement until their SST normalised.

Statistical Analysis

Descriptive statistical analysis was performed on raw data where applicable.

Results

Table 1 shows the demographic/clinical profile of the study population. Twenty-four (39.3%) patients had hypocortisolism, of which 20 (32.8%) had central hypocortisolism as evidenced by concomitant low or inappropriately normal ACTH levels and 4 (6.6%) had primary hypocortisolism with plasma ACTH above the upper reference limit. Of those with central hypocortisolism, 6 received systemic glucocorticoids during SARS while one was an asthmatic on inhaled corticosteroids. The remaining 13 (21.3%) had no prior steroid exposure. One had elevated serum cortisol, ACTH and urinary cortisol, though Cushing's disease was excluded by dexamethasone suppression testing. Among 25 (41%) with HPA axis dysfunction, 15 (24.6%) resolved within a year paralleled by improved well-being, 7 (11.5%) had residual hypocortisolism of which only 1 still required hydrocortisone replacement, whereas 3 defaulted follow-up. There was a definite temporal trend towards improvement in stimulated adrenal cortisol output (Fig. 1). Two of the hypocortisolic cohort had transient subclinical thyrotoxicosis. Four were biochemically hypothyroid, comprising 3 with central hypothyroidism and 1 with primary hypothyroidism. Two of the 3 with central hypothyroidism had concomitant central hypocortisolism.

Discussion

SARS is a new infection with significant morbidity and mortality posing a serious threat to mankind. Theoretically, this coronavirus can involve any organ during the viraemic phase. Because certain forms of hypophysitis, thyroiditis and adrenalitis have viral etiologies,⁴ it is instructive to determine the endocrinopathic impact of SARS-CoV. Recently, researchers have examined the pulmonary and psychological aftermath of SARS survivors.⁵ However, most studies were cross-sectional with limited longitudinal data, and endocrine derangements have not been reported.

Table 1. Clinical Characteristics of the Study Population (n = 61)

Variable	Value
Age	36.5 (25.5 – 47.5)
Gender (male : female)	14 : 47
Race	Chinese (39), Malay (13), Indian (7), Others (2)
Use of corticosteroids during SARS	10
Mechanical ventilation	7
Need for tracheostomy	3

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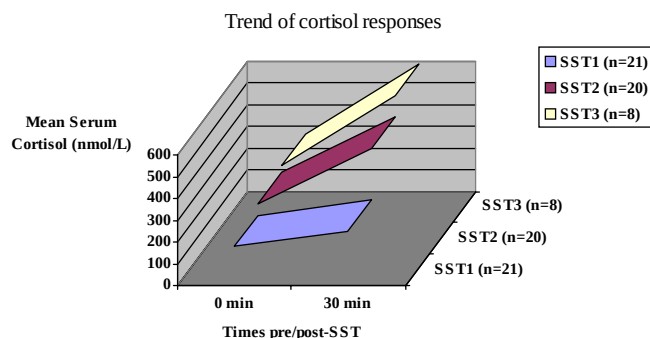


Fig. 1. Results of Short Synacthen test (SST) showing mean serum cortisol responses over a year of follow-up of 21 survivors of SARS SST performed at the first, second and third clinic visit.

Our investigation was an unprecedented effort to chronicle the natural history of endocrinological aberrations in SARS survivors for up to a year. Serial longitudinal evaluation facilitated characterisation of trends and prognostication. A sizeable proportion surprisingly exhibited hormonal deficiencies rather than upregulated HPA axis or thyroid status typical in depression, post-traumatic stress disorder and panic disorder, any of which can occur in SARS survivors. These results provide compelling evidence that the HPA axis and thyroid are candidate targets of SARS-CoV. Central hypocortisolism consequent to a pathological effect of SARS rather than glucocorticoid-induced HPA axis suppression was also suggested by absence of steroid use in nearly two-thirds of this group. Of 5 cases with normal retrospective pre-steroid SST (unpublished ICU data) who received high-dose systemic corticosteroids, 3 with intact HPA axis post-steroids support this postulation. The negative thyroid autoantibodies in all but 1 patient implicate SARS-CoV induced-thyroiditis as a pathogenetic mechanism causing transient subclinical thyrotoxicosis or hypothyroidism. The onset of endocrinopathy in the post-SARS period rather than during acute SARS infection reflects delayed processes possibly mediated at the genomic level.

The prognosis appears favourable as complete resolution occurred in the majority at 1 year. Pending future SARS epidemics, adequately

powered clinical studies to clarify this could prove daunting. In-vitro studies and animal models of SARS might define the endocrine lesions. Predictably, unravelling SARS-cellular interactions and post-receptor molecular cascades could boost drug development to prevent endocrine sequelae. Deciphering SARS-CoV (S1) spike-glycoprotein cross-talk with angiotensin-converting enzyme-2 may serendipitously identify critical pathways nature employs in maintaining endocrine gland integrity.⁶ Translational research in this direction may fuel the design of novel therapeutics to cure endocrine disorders that currently rely on hormonal replacement or suppressive medications for disease control.

Conclusion

Endocrinological perturbations occur frequently in SARS survivors. Although speculative, these findings highlight a possible aetiological role of SARS-CoV as an endocrinopathic factor deserving of future elucidation by in-vitro, animal and clinical studies.

Acknowledgement

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NHG Doctor Award Finalist

Epidemiology of Recurrent Abdominal Pain among Singaporean Adolescents

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Introduction

Recurrent abdominal pain (RAP) is a fairly commonly encountered clinical problem in children. This entity was described by Apley in 1958 when he conducted a field study of 1000 children in the British community. He defined RAP as "paroxysmal abdominal pain occurring in children between 4 and 16 years of age and persisting

for more than 3 months and affecting the child's normal daily activity."¹

The incidence of recurrent abdominal pain has been reported to be 10% to 15% in children between 4 to 16 years of age.² Over the years, we have been seeing an increase in referrals for children with RAP at our hospital. However, there have been no local data about RAP

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in children. The aim of this study was to determine the prevalence and epidemiology of RAP among school-going children in Singapore.

Methods

This study is a cross-sectional survey conducted from June 2003 to October 2003, in Singapore among primary and secondary school students. The study was approved both by the Ministry of Education and the various school administrators. Ten schools were randomly selected using random sampling numbers. Children within each school were randomly selected using cluster sampling.

In addition to information about the students' demographics, socio-economic status and family size, questions were asked about the occurrence and frequency of recurrent abdominal pain, dietary history (e.g., intake of soy milk, vegetables, fruits and milk), stool frequency and common life-event related stresses (e.g., change of school, illness in family and parental job loss). The questionnaires were self-administered by the secondary school students, whilst parents of primary school students were asked to respond to the questionnaires.

Data entry was done by 3 data entry clerks and checked by one of the authors for accuracy and consistency. Data held were password-protected. Statistical analysis was performed using SPSS 12.0. Multivariate logistic regression was performed on the outcome of RAP with the predictors of life, family and school events, dietary and bowel habits and demographics. A forward-stepwise logistic was also performed. Statistical significance was set at $P < 0.05$.

Results

The questionnaire was distributed to 5000 students. Overall response rate was 72% with 3590 students returning complete questionnaires. The incidence of RAP was 23.4% (95% CI, 22%-24.8%). The mean age was 11.7 years (range, 6 to 17 years) of which 62.4% were female. Race distribution was similar to student population distribution with 83% Chinese, 10.3% Malay, 4.8% Indian and 1.9% other races. Significant factors associated with RAP were female sex ($P < 0.001$, OR = 1.7, 95% CI, 1.3-2.1), school work stress ($P = 0.007$, OR = 1.4, 95% CI, 1.1-1.9), changing school ($P = 0.008$, OR = 1.7, 95% CI, 1.2-2.6), parental job loss ($P = 0.007$, OR = 2.1, 95% CI, 1.2-3.5), constipation ($P = 0.002$, OR = 1.8, 95% CI, 1.2-2.5) and number of times admitted to hospital ($P = 0.03$, OR = 1.8, 95% CI, 1.1-3.0).

Discussion

One of the concerns in a self-reported questionnaire study was that patients with undiagnosed organic disease could have been "misclassified" as having functional pain. However, 30.6% of children with RAP in our study had consulted their general practitioners or specialist, and no organic cause had been found. In addition, after excluding all children with constipation from the analysis, there was still a similarly high prevalence of 22.1% children with RAP. We are thus fairly confident that this study is a good reflection of the prevalence of RAP in our local school-going children. This is the first such large-scale epidemiological study in South-East Asia. The only other reported study was performed in rural and urban school children in Malaysia, where Boey et al found a prevalence of 10.2%,³ a much lower figure than what we have found. In another study published in 1995, Hyams described the occurrence of RAP to be approximately 20% of American middle school and high school students, consistent with our belief that there may be a temporal increase in the prevalence of this condition since Apley's study.⁴ It is interesting that this was similar to our local figures suggesting that

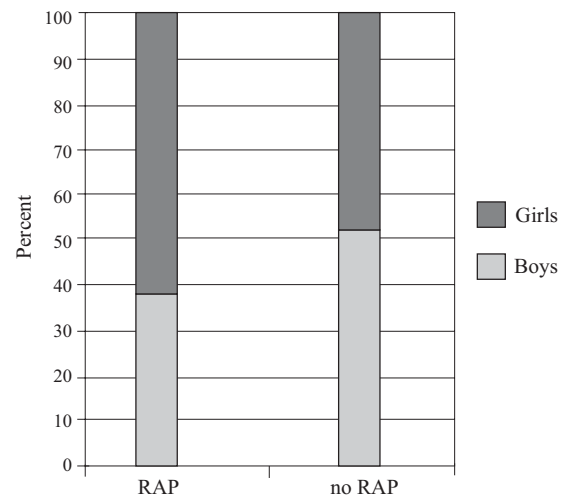


Fig. 1. Sex incidence ($P < 0.001$)

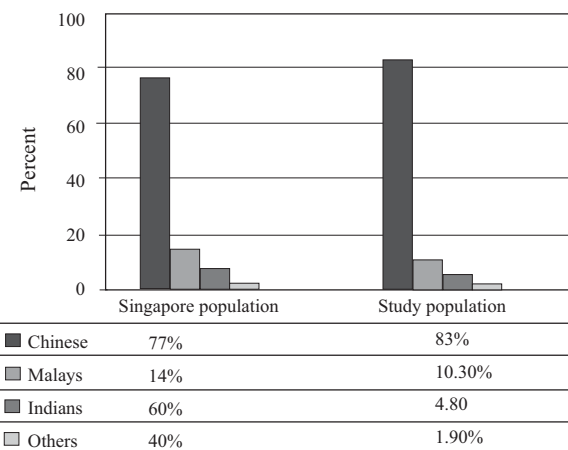


Fig. 2. Racial Incidence

environmental and social factors may play a more important role compared to race and cultural factors in children with RAP.

Conclusion

Our population study determined that the prevalence of RAP in Singapore, an urban developed country in South-East Asia, was 23.4%. This is higher compared to previous reported figures of 10% to 15%. Multivariate analysis found significant associations of RAP with school work stress, changing school, parental job loss, constipation and the number of times the child was admitted to hospital.

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Comparison Between Haematological Parameters in Severe Acute Respiratory Syndrome (SARS) and Dengue Fever (DF)

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Introduction

One of the largest SARS outbreaks occurred in Singapore with most of the patients managed in the SARS-designated Tan Tock Seng Hospital. The cause of SARS has been identified as a novel coronavirus.¹

The WHO clinical case definition of SARS is highly insensitive highlighting the non-specific nature of presenting symptoms.² Although sequencing of the coronavirus genome³ has allowed the development of more specific diagnostic tests, none of these are very sensitive and may not be positive during the first few days of disease.

During the SARS outbreak, tremendous burden was placed on hospitals to provide isolation facilities due to the highly infectious nature of the coronavirus. It would be helpful if some routine laboratory tests could help differentiate SARS from other common infective causes of fever presenting with similar symptoms like DF. This would allow more efficient use of limited isolation facilities.

We studied the use of routine full blood count in discriminating SARS and DF, 2 common causes of febrile illness during the SARS outbreak.

Materials and Methods

SARS cases confirmed by serological testing or identification of viral RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) from March 15 to May 12, 2003 were included. Patients not treated at the SARS-designated hospital, less than 9 years old, or whose history of symptom onset was unclear were excluded. Patients with DF confirmed serologically or by PCR during the same period were included for comparison.

Sequential results of haematological indices including haemoglobin (Hb), white cell (WCC), platelet (PLT), absolute neutrophil (ANC) and absolute lymphocyte counts (ALC) were obtained for each patient and tabulated according to day of illness. The day of symptom onset was designated day 1 of illness.

Longitudinal data analysis technique was applied to detect the

difference between SARS and DF patients in terms of the sequential trend of ALC, ANC, Hb, PLT and WBC levels. The measurements of ALC, ANC, Hb, PLT and WBC in the first 6 days were used for the analysis. In addition, the medians of ALC, ANC, Hb, PLT and WBC levels were also compared for each day by Mann-Whitney U test. Parameters that were significantly different were selected for receiver operator characteristics (ROC) analysis to obtain discriminatory values for the 2 conditions.

Results

Overall, there were 185 and 82 confirmed SARS and DF cases respectively. Of these, the haematological information of 78 SARS patients and 82 DF patients for the first 6 days of illness was available.

SARS patients had significantly lower ALC and Hb and higher ANC and PLT. For some of these parameters, the differences were also significant for the effect of time and interaction between time and groups of patients by longitudinal data analysis (Table 1). The difference in PLT between SARS and DF patients was most marked with a mean difference of $102 \times 10^9/L$ (95% CI, $87 \times 10^9/L$ – $117 \times 10^9/L$). SARS patients also have significantly higher nadir PLT than DF patients (median PLT $151 \times 10^9/L$ versus $34.5 \times 10^9/L$, $P < 0.0001$, Mann-Whitney U test).

Multiple comparisons adjusted by Bonferroni technique and using Mann-Whitney U test showed that ALC was significantly different between SARS and DF from day 3 to day 6, ANC from day 1 to day 5, Hb from day 1 to day 4 and day 6, PLT from day 1 to day 6 and WBC from day 2 to day 3 (Table 1 and Fig. 1).

As PLT and ANC appear to be the most significantly different between the 2 conditions and the difference is most marked in the first 5 days, we decided to subject these 2 parameters during the first 5 days to ROC analysis to find the best discriminatory value between the 2 conditions. The results showed that PLT is a better discriminator than ANC and day 2 and day 3 values are most useful. Using the mean

Table 1. Comparison of Sequential and Daily Haematological Parameters between SARS and DF

Parameters	SARS vs DF(Sequential)	Mean difference	Effect of time	Interaction between time and patient group	SARS vs DF(Daily)
ALC	SARS Lower($P < 0.0001$)	$0.79 \times 10^9/L$ (95% CI, 0.62 – $0.92 \times 10^9/L$)	$P < 0.0001$	$P < 0.0001$	D1 ($P = 0.622$)D2 ($P = 0.01$) D3 to 6 ($P < 0.0001$)
ANC	SARS Higher($P < 0.0001$)	$1.62 \times 10^9/L$ (95% CI, 1.18 – $2.06 \times 10^9/L$)	$P = 0.24$	$P < 0.0001$	D1 to 5 ($P < 0.0001$) D6 ($P = 0.012$)
Hb	SARS Lower($P < 0.0001$)	1.08 g/dL (95% CI, 0.62 – 1.53 g/dL)	$P < 0.0001$	$P = 0.0025$	D1 to 3 ($P = 0.002$)D4 ($P = 0.005$) D5 ($P = 0.132$)D6 ($P = 0.004$)
PLT	SARS Higher($P < 0.0001$)	$101.9 \times 10^9/L$ (95% CI, 86.9 – $117.0 \times 10^9/L$)	$P < 0.0001$	$P < 0.0001$	D1 to 6 ($P < 0.0001$)
WBC	No Significant Difference($P = 0.089$)	-	$P = 0.142$	$P < 0.0001$	D1 ($P = 0.012$)D2 ($P < 0.0001$) D3 ($P = 0.002$)D4 ($P = 0.919$) D5 ($P = 0.154$)D6 ($P = 0.014$)

ALC: absolute lymphocyte counts; ANC: absolute neutrophil count; DF: dengue fever; Hb: haemoglobin; PLT: platelet; SARS: severe acute respiratory syndrome; WBC: white blood count

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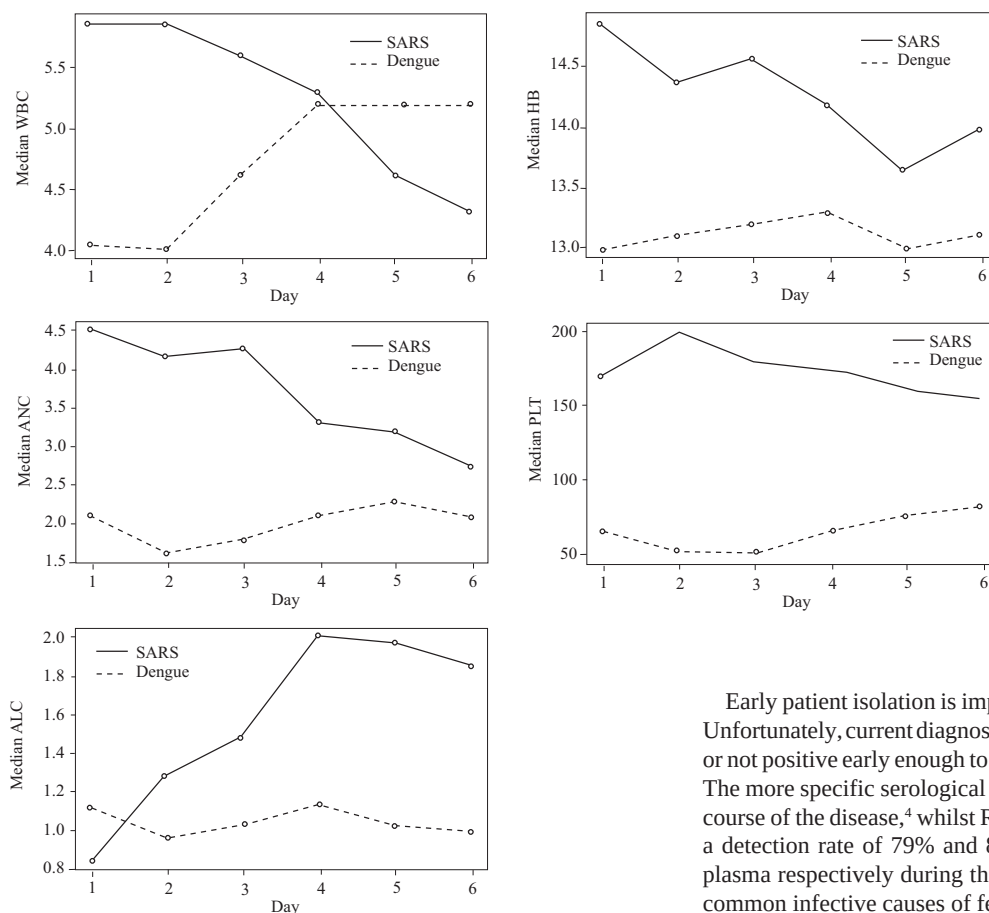


Fig. 1. Trends in different haematological parameters during the first 6 days of illness for SARS and dengue patients.

of day 2 and day 3 PLT as the predictor, the area under ROC curve is 0.976 (95%CI, 0.95-1). The best cut-off PLT value is $123.5 \times 10^9/L$ and this will differentiate SARS from DF with a sensitivity of 95.9% and specificity of 92%. Using logistic regression with the mean of day 2 and day 3 PLT and the mean of day 2 and day 3 ANC as the predictors, a predicted probability of SARS is calculated for each patient, and the predicted probability is used for ROC analysis. The area under ROC curve is only improved to 0.978 (95% CI, 0.952-1) but the interpretation will be difficult because the cut-off point will be using predicted probability calculated from the model and this cut-off point is not easy to use in practice. So the mean of day 2 and day 3 platelet count alone is probably good enough as a discriminator between these 2 common causes of febrile illness during a SARS outbreak.

Discussion

During a SARS outbreak, most patients with fever presented themselves to the emergency department at Tan Tock Seng Hospital. This gave us the opportunity not only to study the sequential changes in haematological parameters in SARS but also other common infections like DF.

Early patient isolation is important for the containment of SARS. Unfortunately, current diagnostic tests are either not sensitive enough or not positive early enough to allow identification of SARS patient. The more specific serological test only becomes positive late in the course of the disease,⁴ whilst RT-PCR for the coronavirus RNA has a detection rate of 79% and 80% in nasopharyngeal aspirate and plasma respectively during the first 3 days of illness.^{5,6} One of the common infective causes of febrile illness in Singapore is DF. Our study showed that using a cut-off platelet count of $123.5 \times 10^9/L$ on day 2 and day 3 of febrile illness, SARS could be differentiated from dengue with a sensitivity and specificity of greater than 90%.

Conclusion

Platelet count early on in the disease allows for differentiation between 2 common infective causes of febrile illness during a SARS outbreak and may facilitate more effective utilisation of isolation facilities.

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Prevalence of Dementia in Singapore – Results of the National Mental Health Survey of the Elderly 2003

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Background/Aims

Singapore's rate of population ageing (3% annually) is 2 to 3 times greater than those in developed countries. The population comprises of 7.6% elderly people ≥ 65 years old. The three main ethnic groups are the Chinese (77%), Malay (14%) and Indians (8%).

Two major surveys were previously done in Singapore to determine the prevalence of dementia in Singapore. The first survey done in 1985 involved 612 Chinese living in the community, using the Geriatric Mental State Examination (GMS),^{1,2} found the prevalence of dementia to be 1.8%.³ Another survey done in 1990 involving 349 elderly subjects, using the Elderly Cognitive Questionnaire (ECAQ) to screen, followed by the GMS, found the prevalence of dementia to be 4.0% in Malay elderly and 2.5% in Chinese elderly.⁴ A study on 234 elderly Chinese living in the Queenstown district in 2002 found the prevalence of cognitive impairment to be 7.7% using the ECAQ and 13.2% by the Informant Questionnaire on the Cognitive Decline of the Elderly (IQCODE).⁵

This study aims to provide nationally representative estimates of the prevalence of dementia in the elderly population of Singapore.

Method

A cross-sectional community survey of a national ethnically stratified random sample of older adults 60 years and above ($n = 1092$) was conducted. Selection of households is culled from MOH's census of addresses in Singapore. As close to an equal number of clients per district as possible was recruited. The criteria for inclusion into the study were: 60 years or above; citizens or permanent residents of Singapore; Chinese, Malay and Indian ethnicity; must not be incapacitated or mentally unfit to answer the interview questions. Domestic workers and sub-tenants were not qualified for the interview. The subjects were interviewed in their homes by Chinese, Malay or Indian psychiatric nurses who have been trained to administer the GMS. Chinese and Malay translated versions of the GMS were used for interviewees who could not understand English. A pilot study involving 100 elderly was done before the actual survey. Socio-demographic data was collected and the GMS^{3,4} was administered. The Automated Geriatric Examination for Computer Assisted Taxonomy (AGECAT) was used to diagnose dementia.

Results

The response rate was 72.4%. The non-response rate was 27.6%. The number of cases who could not be contacted was 345 (22.9%). The number who was rejected was 71 (4.7%). The final number in the sample was 1092. The overall prevalence of dementia for older adults ≥ 60 years, ≥ 65 years and ≥ 75 years were 5.2%, 6.0% and 13.9% respectively. The prevalence of dementia in the age groups 60-64 years, 65-74 years, 75-84 years and ≥ 85 years were 0.8%,

Table 1. Population-weighted Prevalence Point Estimates (95% Confidence Intervals) of Dementia in Older Adults Aged 60 and Above in Singapore, 2003

	N	%	(95% CI)
≥ 60 years (overall)	77	5.2	(3.6-6.7)
≥ 65 years	73	6.0	(4.0-8.0)
≥ 75 years	40	13.9	(7.9-19.9)
Age			
60-64 years	4	0.8	(0.0-1.8)
65-74 years	33	4.0	(2.0-5.9)
75-84	28	9.2	(4.5-13.9)
≥ 85 years	12	32.2	(11.7-52.7)
Gender			
Male	16	2.2	(0.7-3.6)
Female	61	7.8	(5.1-10.4)
Ethnicity			
Chinese	22	4.2	(2.5-6.0)
Malay	34	9.4	(6.2-12.6)
Indian	21	8.8	(5.0-12.5)

There were statistically significant differences in dementia prevalence by gender ($P < 0.001$), age ($P < 0.001$), and ethnicity ($P < 0.001$).

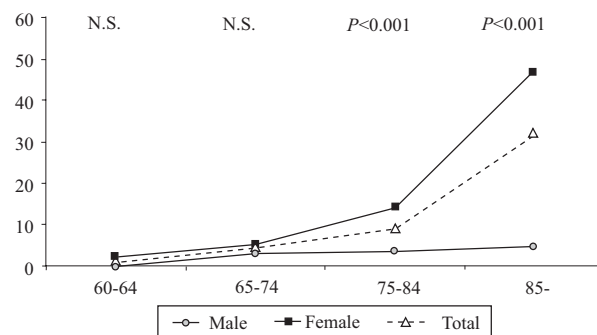


Fig. 1. Prevalence of dementia by gender and age.

4.0%, 9.2% and 32.2% respectively. Among elderly ≥ 65 years old, the prevalence of dementia in male and female subjects was 3.2% and 8.3% respectively ($P < 0.001$); in Chinese, Malay and Indian ethnic groups it was 5.2%, 11.1% and 7.2% respectively ($P < 0.001$).

Conclusion

These are the first estimates of dementia prevalence based on a nationally representative sample of elderly subjects in Singapore. They are twice as high as those determined from limited sample data from selected districts a decade ago, and point to a need for current review and future planning of resources to provide for adequate and effective care and management of the cognitively impaired/demented elderly in a rapidly ageing community.

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NHG Doctor Award Finalist

Preoperative Microalbuminuria, Haptoglobin Phenotype 2-2, and Age Are Independent Predictors for Acute Renal Failure Following Coronary Artery Bypass Graft

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Introduction

Acute renal failure (ARF) after coronary artery bypass graft (CABG) is associated with high morbidity and mortality. Microalbuminuria (MAU) and haptoglobin phenotype were recently reported to be associated with mortality in patients with ischaemic heart disease. We hypothesised that the presence of pre-op MAU and an Hp2-2 phenotype may also be independent risk factors for post-CABG ARF.

Materials and Methods

Over 2 years, 148 patients referred for elective isolated CABG by their cardiologists were recruited for this prospective study at National University Hospital (Singapore). ARF was defined as a rise in serum creatinine (cr) level of ≥ 0.5 mg/dL when baseline cr was < 2.0 mg/dL or ≥ 1.5 mg/dL when baseline was ≥ 2.0 mg/dL. MAU was defined as a 24-hour urine albumin excretion of 30 mg to 300 mg. Hp was determined by non-denatured polyacrylamide gel electrophoresis (PAGE) and peroxidase staining.

Results

Out of 148 patients (male gender 80%; age: 59.7 ± 8.7 yrs; Chinese: 70.3%, Malay: 12.8%, Indian: 16.9%; type 2 DM: 60%), 27 patients developed postoperative ARF (18.2%). Three patients (2%) required dialysis (1 Hp2-1 and 2 Hp2-2) and 3 patients (2%) (3 Hp2-2) died. On univariate analysis, ARF was significantly associated with age (65.6 ± 6.5 years versus 58.4 ± 8.6 years, $P = 0.0001$), lower preoperative ejection fraction (EF) ($36 \pm 15\%$ versus $45 \pm 13\%$, $P = 0.007$), Hb (12 ± 2 versus 14 ± 1 mg/dL, $P = 0.006$) and 24 h urine creatinine clearance rate (59 ± 23 versus 73 ± 24 , $P = 0.01$), higher preoperative creatinine level (12 ± 36 versus 102 ± 36 $\mu\text{mol/L}$, $P = 0.01$) and polymorphic WBC percentage ($63 \pm 7\%$ versus $59 \pm 9\%$, $P = 0.009$), preoperative left ventricular (LV) enlargement (30% versus 12%, $P = 0.008$) and Digoxin use (67% versus 15%, $P = 0.001$). Hp2-2 (25% Hp2-2 versus 11% Hp1-1 and Hp2-1 combined,

$P = 0.03$) and MAU were also significantly associated with ARF (55% MAU versus 8% non-MAU, $P = 0.0002$). On multivariate analysis preoperative MAU (OR = 56, 95% CI, 7-445, $P = 0.0003$), Hp2-2 (OR = 5.4, 95% CI, 1.2-24, $P = 0.03$) and age ≥ 60 years (OR = 18, 95% CI, 3-126, $P = 0.003$) remained as independent predictors for ARF after adjusting for gender, race, diabetes, preoperative LV enlargement, digoxin use, Hb level, EF and creatinine level (Table 1).

Table 1. Multivariate Analysis of Factors Associated with Acute Renal Failure Following Elective Isolated Coronary Artery Bypass Graft

Covariate	OR	95% CI for OR	P value*
Race [Malay vs (Chinese + Indian)]	6.3	0.8-42	0.08
Gender (Female)	1.4	0.2-9.9	0.73
Preoperative Hb ($< 12\text{g/dL}$)	1.3	0.2-7.2	0.75
Preoperative LV enlargement (Yes vs No)	3.6	0.8-17	0.1
Preoperative EF ($< 45\%$)	1.04	0.2-5.2	0.96
Diabetes (Yes vs No)	0.2	0.04-1.2	0.08
Preoperative digoxin usage (Yes vs No)	2.0	0.2-1.2	0.06
Preoperative serum creatinine ($\geq 120\text{ }\mu\text{mol/L}$)	3.3	0.6-19	0.19
Age (≥ 60 y)	18	3-126	0.003
Hp phenotype [Hp2-2 vs (Hp1-1+Hp2-1)]	5.4	1.2-24	0.03
Preoperative microalbuminuria (Yes vs No)	56	7-445	0.0003

*P values from Wald test.

Discussion

Proteinuria has been accepted as a reliable indicator of the severity of end-stage renal disease. Microalbuminuria (MAU) is defined as the presence of urinary albumin excretion of 30 to 300 mg/24 h. MAU is a clinical marker for early kidney dysfunction. The presence of MAU defines the clinical onset of diabetic nephropathy. MAU has also been previously reported to be a powerful predictor of cardiovascular disease in both diabetic and nondiabetic subjects.^{1,2} Our finding of MAU as the strongest predictor of ARF following CABG, independent of such known variables such as diabetes,

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hypertension and pre-existing renal dysfunction, is novel and unexpected. The underlying basis for this association remains unclear. We hypothesise that MAU may be a clinical marker for small vessel disease in the kidney, which is likely to be common in high-risk patients with large vessel atherosclerotic disease (e.g., coronary artery disease) requiring CABG surgery. It is likely that patients with small vessel disease of the kidney are more vulnerable to the decrease and maldistribution of renal blood flow, the increase in renal vascular resistance, and the decrease in glomerular filtration rate that occur during CABG surgery.

Haptoglobin (Hp), an α_2 -sialoglycoprotein with haemoglobin (Hb)-binding capacity, has 3 phenotypes: Hp1-1, Hp2-1 and Hp2-2. The Hb-binding function of Hp prevents iron loss and has until recently been generally accepted as the major mechanism underlying prevention of kidney injury ("pigment nephropathy") during haemolysis. However, recent studies show that Hp appears to play an important physiological role as an antioxidant rather than an Hb-binding protein which prevents renal deposition of Hb during haemolysis. The known ability of Hp to inhibit the synthesis of vasoconstricting prostaglandins may underlie the renal vasoconstriction leading to ARF in this mouse model of chemically-induced haemolysis.^{3,4} Hp 2-2 has been reported to be associated with the prevalence of autoimmune and inflammatory disorders, as well as cardiovascular conditions such as essential hypertension, CAD mortality, and restenosis after PTCA.^{3,5} During CABG surgery, the requirement for intraoperative cardiac pulmonary bypass is associated with haemolysis. We now report the novel identification of Hp 2-2 as an independent risk factor for the development of ARF following CABG.

A steady decline in renal function is a normal event associated with ageing. The ageing process results in anatomic and functional

changes in the kidney. Histological examination confirms the disappearance of functional nephrons with age; as many as half of the glomeruli present in young adults may be gone or rendered nonfunctional by 80 years of age.⁶ The common functional change is a diminution in renal reserves, along with constraints on the kidney's ability to respond appropriately to acute illness. Consistent with previous studies of ARF following CABG,^{7,8} we found that age ≥ 60 years is an independent risk factor for ARF in our study.

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Proteomics of Breast Cancer: Enhanced Expression of CK19 in HER-2/*neu*-positive Tumours

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Introduction

Breast cancer development is a complex process which involves complicated and interlinked molecular events caused by the altered expression of both oncogenic and tumour suppressor genes and the related signal pathways. HER-2/*neu* is one of the important oncogenes in breast cancer encoding a transmembrane tyrosine kinase receptor responsible for signalling cascade activation. Its overexpression indicates an adverse prognosis effect with a more aggressive phenotype and shortened overall survival rates.

Overexpression of HER-2/*neu* is also consistently associated with high tumour grade, DNA aneuploidy, high cell proliferation rate, p53 mutation, topoisomerase II α amplification and alterations of other molecular biomarkers of breast cancer invasiveness and metastasis.¹ However, the biological basis by which HER-2/*neu* overexpression confers the characteristically more aggressive breast tumour proliferation, invasion and metastasis associated with such tumours remains to be elucidated.

In this study, we aim to identify differentially expressed proteins which are potentially involved in the more aggressive phenotype of the subset of breast cancer associated with the HER-2/*neu* abnormality.

Materials and Methods

The breast cancer tissues which had been previously categorised as either HER-2/*neu* positive or HER-2/*neu* negative by both FISH and IHC² were obtained from the tumour bank of the National University Hospital of Singapore. Laser capture microdissection (LCM) was used to procure unique cell populations and 2-dimensional gel electrophoresis (2-DE), to separate the proteins between the 2 subtypes of tumours.

The differentially expressed proteins were identified using NCBI protein database, and Mascot software based on the peptide mass fingerprinting data obtained from MALDI-ToF MS analysis.

Western blot, reverse-phase protein array and immuno-histochemistry on tissue microarray sections were performed to further confirm the differential expression levels of the identified proteins in the two tumour subtypes. Semi-quantitative RT-PCR was carried out to validate the differential mRNA transcription.

Results

Seven differentially expressed proteins were unambiguously identified. Five proteins (tropomyosin 3, cytokeratin 19 [CK19], cathepsin D, aldolase A and glyoxalase I) were found to be overexpressed, whereas 2 proteins (MnSOD and serum albumin) were down-regulated in the HER-2/*neu*-positive breast tumours (Fig. 1). CK19 is a structural component of the epithelial cytoskeleton that plays an important role in cell migration and invasion. Its overexpression in the cohort of HER-2/*neu*-positive breast tumours was further analysed by reverse phase protein array and RT-PCR. As shown in Figure 2, the CK19 mRNA level in HER-2/*neu*-positive tumour cells was significantly higher compared to that in HER-2/*neu*-negative cells ($P = 0.036$). Immunohistochemical analysis on

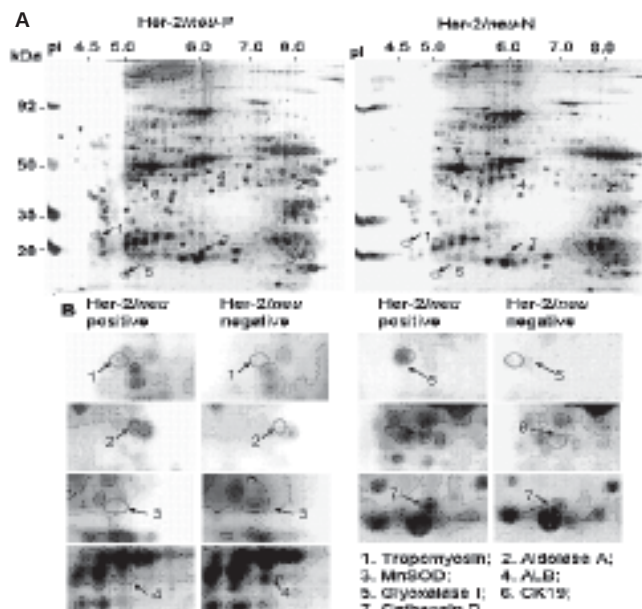


Fig. 1a. 2-D gels derived from laser-microdissected breast tumours. The pooled HER-2/*neu*-positive and pooled HER-2/*neu*-negative laser-microdissected cells were lysed and run on IPG DryStrip (pH3-10 NL). Proteins were further separated by SDS-PAGE (8.5%) gels and silver-stained. Seven differentially expressed proteins were identified by MALDI-TOF MS analysis.

Fig. 1b. Enlarged view of the 7 spots differentially expressed between HER-2/*neu*-positive and -negative breast tumour cells

tissue microarray sections comprising 97 breast tumours revealed that CK-19 was strongly stained in 81.5% of HER-2/*neu*-positive tumours and in 48% of HER-2/*neu*-negative tumours ($P = 0.0043$).

Discussion

The importance of proteomics techniques for clinical and diagnostic cancer research has been repeatedly addressed in the development of potential biomarkers and the documentation of the marked changes in the protein profiles during the various stages of cancer growth, invasion and metastasis. HER-2/*neu* overexpression is strongly associated with a poor prognosis and an increased likelihood of metastasis, and thus identification of the differentially expressed proteins in HER-2/*neu*-dependent breast cancer is crucial to elucidate the mechanisms of tumorigenesis. Using proteomics, we identified 2 structural proteins (tropomyosin 3, CK 19), and 3 cellular metabolic proteins (aldolase A, glyoxalase I, cathepsin D) which were overexpressed in HER-2/*neu*-positive tumours, whereas MnSOD and serum albumin were down-regulated.

Cytokeratins are intermediate filaments whose expression are often altered in epithelial cancer and are involved in the structural organisation of the cells. Differential expression of CK7, CK8, CK17, CK18 and CK19 have been reported in breast cancer and

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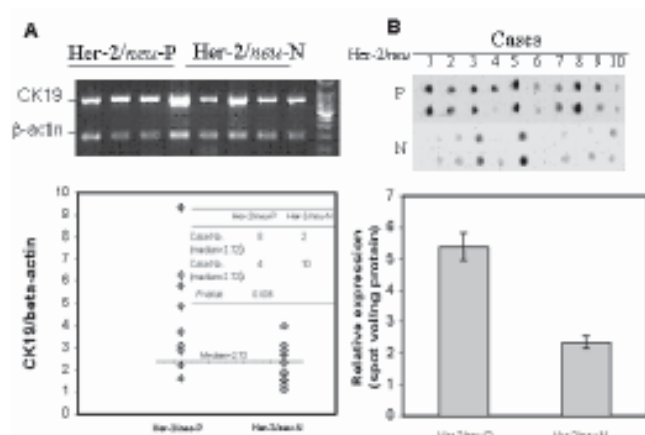


Fig. 2a. RT-PCR analysis of CK19 expression. A representative RT-PCR from 4 HER-2/neu-positive and 4 HER-2/neu-negative tumour cells was shown (top). Median of CK19 expression in 24 different LCM cells was calculated using SPSS 12.0 software and the P-value was analysed by Fisher's exact test (bottom).

Fig. 2b. Reverse-phase protein arrays from 10 HER-2/neu-positive and 10 HER-2/neu-negative laser-microdissected tumour cells. Duplicated samples were manually spotted onto PVDF membrane (top) and arrays were probed with CK19 Ab-1 and signals were detected as described in B. Relative expression of CK19 in each sample was normalised to the signal intensity of β -actin.

other cancers. CK19 expression was found to increase proportionally with the metastatic potency of hepatocellular carcinoma (HCC) cell lines and in relation to poor differentiation.³ A significantly higher incidence of early tumour recurrence and/or extrahepatic disease was found in patients with CK19-positive HCC than in those with CK19-negative HCC, and was attributed to the increased invasiveness of the former.⁴ Serum levels of CYFRA 21-1, a CK 19 fragment, have been used as a tumour marker in a variety of cancers, such as non-small-cell lung cancer,⁵ to detect occurrence of metastasis and tumour cell dissemination. In this study, we found that CK19 expression is unambiguously related to the HER-2/neu status in breast cancer. Overexpression of CK19 in most HER-2/neu-positive breast cancer is likely to increase the probability of metastasis and confer unfavourable prognosis, as found in (HCC) cell lines.³

The other proteins we identified, namely, tropomyosin 3, aldolases A, glyoxalase I, and cathepsin D, are also highly likely candidates implicated in the heightened aggressiveness of HER-2/neu-positive breast tumours. Tropomyosin 3 transfection is reported to induce lamellipodial formation, increase cellular migration and reduce stress fibres.⁶ Aldolase A is a key enzyme in glucose metabolism where it promotes cell growth when overexpressed. Glyoxalase I

catalyses the detoxification of alpha-oxoaldehydes to corresponding aldonic acids, thus protecting cells from toxic damage and inducing a pro-survival response to chemotherapy. Cathepsin D is a protease involved in protein metabolism and tissue remodelling which might support the cell proliferation and stimulate invasion. The high expression of these metabolic enzymes may explain, at least in part, the more progressive phenotype and adverse clinical outcome in patients with HER-2/neu-positive tumours.

In contrast, we found that serum albumin and MnSOD were up-regulated in HER-2/neu-negative breast tumours. Overexpression of albumin was also reported in HER-2/neu-negative infiltrating ductal carcinoma, with unknown mechanism. MnSOD protects cells from oxidative stress by catalysing the dismutation of superoxide ($O_2^{\cdot -}$) radicals into hydrogen peroxide (H_2O_2). Our data may suggest that other protective molecules against oxidative damage, instead of other than MnSOD, may against oxidative damage be overexpressed in HER-2/neu-positive tumour cells.

Conclusion

Collectively, our results demonstrated that HER-2/neu-mediated rapid tumour growth and metastasis is a complicated process resulting from, or leading to, the imbalance of cell structural organisation, overall metabolism and resistance to stress. Arguably, proteome analysis will provide the best approach to achieve a fundamental understanding of the HER-2/neu-related network of metabolic and structural molecules involved in the formation and progression of breast cancer, and to move closer to the target of discovering protein biomarkers for cancer diagnosis, prognosis and therapy.

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Celecoxib Enhances Brain Tumour Cell Radiosensitivity Leading to Massive Tumour Necrosis

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Introduction

Radiotherapy remains integral for the treatment of glioblastoma patients who typically survive less than 18 months despite standard therapy of surgery, radiotherapy and chemotherapy. Cyclooxygenase-2 (COX-2), a rate-limiting enzyme in conversion of arachidonic acid into prostaglandins, is overexpressed in irradiated tumour cells, which is associated with radioresistance.¹ With the increasing evidence of COX-2 contributing to tumour growth and converse tumour regression following treatment with COX-2 inhibitors,^{2,3} there is potential therapeutic benefit to enhance tumour radioresponse with COX-2 inhibitors. Indeed, several reports have shown enhanced tumour radiosensitivity with various COX-2 inhibitors in vitro and in vivo.^{4,5} Nevertheless, in brain tumours, only 1 study showed enhanced tumour radioresponse to SC-236, a selective COX-2 inhibitor, in U251 human glioblastoma cells.³ To date, the underlying mechanisms responsible for enhanced tumour radiosensitivity by COX-2 inhibitors remain unclear, although several mechanisms have been proposed (e.g., induction of cell apoptosis, cell cycle arrest, sublethal radiation damage and anti-angiogenesis). In this study, we determined whether the selective COX-2 inhibitor celecoxib, could enhance brain tumour radiosensitivity in vitro and in vivo, by using U87 human glioblastoma cells grown as monolayer and transplanted intracranially into nude mice. To elucidate whether this effect is due to damaged microvessels and anti-angiogenesis mechanisms, we determined the combined effect of celecoxib and irradiation by measuring tumour necrosis and microvascular density of mice brain tumours in vivo, as well as the expression of angiogenic factors vascular endothelial growth factor (VEGF), angiopoietin-1 and angiopoietin-2 proteins of U87 cells in vitro.

Materials and Methods

In vitro, U87 human glioblastoma cells were treated with celecoxib (30 mmol/L), irradiation (9 Gy), combined celecoxib and irradiation, or vehicle dimethyl sulfoxide (DMSO) for 24, 48 and 72 hours, and cell viability was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. After 72 hours of treatment, COX-2, VEGF, angiopoietin-1 and angiopoietin-2 proteins were quantitatively analysed by Western blot, whereas prostaglandin E₂ in the culture medium was measured by enzyme immunoassay.

In vivo, 36 nude mice were intracranially transplanted with U87 cells (5000 cells in 5 µL medium). After 7 days, the mice were treated with celecoxib (100 mg/kg for 2 weeks), irradiation (8 Gy for 3 days), combined therapy or vehicle DMSO, and the survival time recorded. Upon death, brain tumours were isolated, fixed, paraffin-embedded, coronally sectioned (4 µm) and stained with haematoxylin and eosin. The largest tumour region was measured to calculate brain tumour volume, by 0.5 x width² x length formula. For necrosis analysis, the largest tumour necrotic region was measured, and presented in percentage of necrosis over largest tumour area. For microvascular density, adjacent tissue of the largest tumour section was

immunohistochemically stained with von Willibrand factor endothelial marker. Any positive-stained endothelial cell, or endothelial-cell cluster that was separated from tumour cells and other connective tissue elements was considered as a single, countable microvessel. All measurements were aided by Zeiss KS400 software, and performed by 2 independent observers in a blinded manner. The intra- and inter-variability of these observers were validated by simple regression analysis.

GBSTAT School Pak statistical package was used in all statistical analysis. The mice survival curves were analysed by Logrank survival test. Treatment effects on cell viability, proteins, prostaglandin E₂, necrosis and microvascular density were analysed by 1-way analysis of variance (ANOVA)-Dunnnett's test, and differences between 2 treatment groups analysed by Student's *t*-test. Probabilities less than 0.05 (*P* < 0.05) were considered to be statistically significant.

Results

Irradiation and celecoxib independently caused significant reduction (10% to 20%, and 36% to 40% respectively) in U87 cell viability at 24, 48 and 72 hours. When combined with irradiation, celecoxib significantly reduced cell viability by 54% to 63%, compared to controls (Fig. 1A). At 72 hours, COX-2 protein was increased by celecoxib and/or irradiation (Table 1A). The COX-2 metabolite, prostaglandin E₂ was increased by irradiation alone at 72 hours. However, this increase was completely blocked with the presence of

Table 1. The Effect of Celecoxib and/or Irradiation on Protein Expression (COX-2, VEGF, angiopoietin-1, angiopoietin-2) and Prostaglandin E₂ in vitro, as well as Tumour Necrosis and Microvascular Density in vivo

	Control	Irradiation	Celecoxib	Combination
A. In vitro COX-2	0.8 ± 0.2	1.6 ± 1.3**	1.4 ± 0.3*	1.7 ± 0.3*
Prostaglandin E ₂ (ng/mg total protein)	208.0 ± 51.1	684.7 ± 79.4**	3.7 ± 0.5**	5.4 ± 0.5**
VEGF	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.1**	0.8 ± 0.1*
Angiopoietin-1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1*	0.4 ± 0.1*
Angiopoietin-2	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1*	0.8 ± 0.1**
B. In vivo				
Necrosis/ tumour area (%)	2.2 ± 1.4	1.4 ± 1.1	8.1 ± 3.7*	23.3 ± 8.5** #
Microvessels/mm ²	91.2 ± 7.2	65.4 ± 4.0*	66.4 ± 4.8*	52.5 ± 2.9** #

COX-2: cyclooxygenase-2; VEGF: vascular endothelial growth factor

Table 1. Protein levels (analysed by Western blot) were presented in arbitrary units normalised against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein. Prostaglandin E₂ was measured by enzyme immunoassay. The largest tumour necrotic region was presented in percentage over largest tumour area. Microvascular density was analysed by immunohistochemical staining of von Willibrand Factor on non-necrotic tumour region. All data are expressed in mean ± standard error of mean, representative of 4 to 8 independent experiments in vitro or 8 to 10 mice in vivo. **P* < 0.05, ***P* < 0.01 significantly different from control groups (1-way ANOVA, Dunnnett's test). #*P* < 0.05 significantly different between combination and celecoxib-treated groups (Student's *t*-test).

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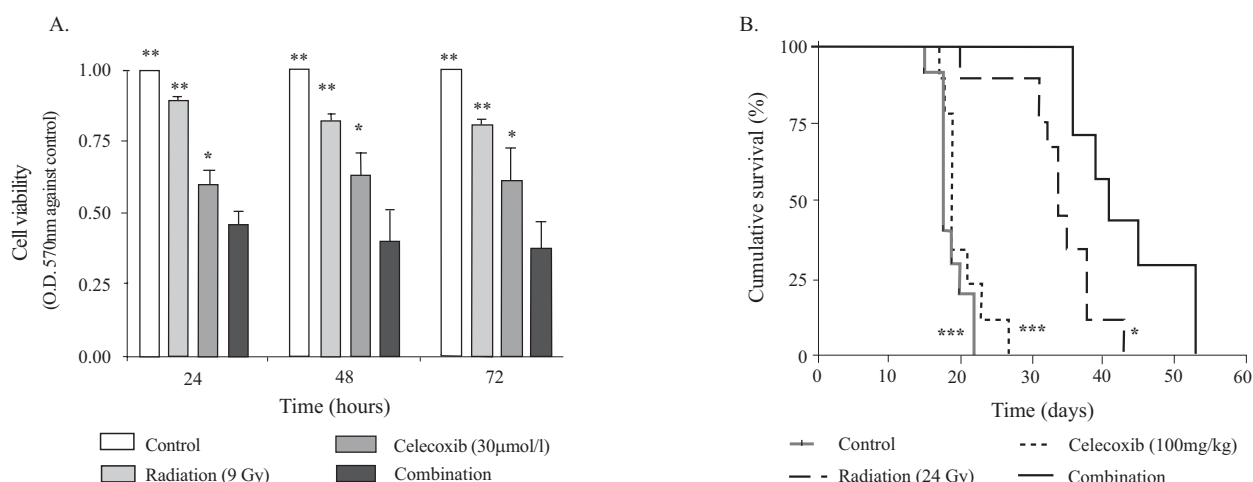


Fig.1. Celecoxib enhances brain tumour radiosensitivity in vitro and in vivo. The effect of celecoxib, irradiation, combined celecoxib and irradiation, or DMSO (as control) on U87 cell viability (Fig. 1A) and the median survival of mice intracranially transplanted with U87 cells (Fig. 1B), are shown. Each group represents 7 to 8 independent experiments (Fig. 1A) or 8-10 mice (Fig. 1B). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ significantly different from combined therapy group (Fig. 1A: 1-way ANOVA, Dunnett's test; Fig. 1B: Logrank survival test).

celecoxib (Table 1A). When combined with irradiation for 72 hours, celecoxib reduced VEGF and angiopoietin-1, but increased angiopoietin-2 proteins (Table 1A).

Irradiation extended the median survival of brain tumour mice from 18 (controls) to 33 days. Combined celecoxib and irradiation further extended the median survival to 41 days (Fig. 1B). Upon death, the average brain tumour volume of all mice was 45.2 ± 3.9 mm³. Celecoxib-treated tumours contained significantly greater proportion of necrosis compared to irradiated and control tumours. Irradiation further increased necrosis of celecoxib-treated tumours by approximately 190% (Table 1B), validated by 2 independent observers with intra- and inter-variability correlation coefficient values of 0.99. Single therapy of irradiation or celecoxib significantly reduced tumour microvascular density (compared to controls), which was further reduced by combination therapy (Table 1B). Two independent observers validated these data with intra- and inter-variability correlation coefficient values of 0.98 and 0.90, respectively.

Discussion

Celecoxib significantly increased brain tumour radiosensitivity, as demonstrated by reduced U87 cell viability and extended survival of brain tumour mice. There is 1 study, which showed enhanced brain tumour radioresponse by SC-236 in U251 human glioblastoma cells in vitro and as xenograft in mice thighs.³ Our novel study used celecoxib (currently prescribed for pain and inflammation with established safety profile), on U87 human glioblastoma cells in vitro and intracranially transplanted into mice brain, which mimics a direct microenvironment of brain tumours in vivo. Moreover, our

study used celecoxib 100 mg/kg/day in mice, which produced peak plasma concentration of 3 to 4 mmol/L,⁶ equivalent to human plasma concentration after 800 mg/day celecoxib, a Food and Drug Administration-approved dosage.

In defining the underlying mechanism, we showed massive tumour necrosis and inhibition of angiogenesis by celecoxib to enhance brain tumour radioresponse. In addition to tumour cells, combined celecoxib and irradiation targets and destroys the tumour microvasculature, thus causing massive necrosis. This may be due to celecoxib reduction of prostaglandin E₂, which is known to be pro-angiogenic and pro-proliferative, thus a survival factor in irradiated tumours. Celecoxib also reduced the expression of other pro-angiogenic factors VEGF and angiopoietin-1, and increased angiopoietin-2 proteins, an antagonist of angiopoietin-1. Our findings are novel and with the proven safety profile of celecoxib, these results can potentially be translated clinically to glioblastoma patients.

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In Vitro Drug Sensitivity and Expression Profiling For Disease Prognostication in Childhood Acute Lymphoblastic Leukaemia (ALL): An Exploratory Model Using Cell Lines

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Introduction

Poor prednisolone response, defined as the peripheral blast count $\geq 1,000/\mu\text{L}$ after 7 days induction with prednisone and 1 dose of intrathecal methotrexate, defines a high-risk group of patients with poor outcome (~38% to 50% EFS compared to 80% EFS in prednisolone good responders).^{1,2} Many treatment protocols, including ours and the widely adopted German Berlin-Frankfurt-Munster clinical trials, utilise prednisolone response as an important prognostic factor. This suggests that resistance to therapy is already present at initial diagnosis and this can be exploited to improve prediction of eventual outcome.

Global gene expression profiling (GEP) using the microchip array provides a snapshot of the messenger RNA transcripts of the cells and hence divulges the pathways active in the cell. Although GEP can be used to diagnose and subgroup many different types of cancer including acute lymphoblastic leukaemia,³ it has been unable to prognosticate who is able to do well compared to those who will relapse. We postulate that the leukaemia cells at diagnosis have no need to manifest their resistance pathways as this confers no survival advantage. This may explain why GEP at diagnosis is not helpful in prognostication. However, we suspect that, paired GEP of leukaemia cells at diagnosis and after therapy with prednisolone may unmask the resistance pathways as prednisolone therapy alone is a powerful prognostic factor.

Our aim is to identify and compare the changes in GEP of leukemic cells prior to, and after, exposure to prednisolone to define a molecular signature for prognosis. We started this proof of concept using the leukaemia cell lines.

Materials and Methods

Cell Lines

Four clinically important ALL cell lines were studied: Reh/TEL-AML1 t(12; 21), 697/E2A-PBX1 t(1; 19), Sup-B15/BCR-ABL t(9; 22) and RS4; 11/MLL-AF4 t(4; 11). All cell lines were purchased from the ATCC and cultured according to the ATCC's instructions.

MTT Assay

Prednisolone 21-hemisuccinate sodium salt was serially diluted to 6 concentrations (0.08 to 250 $\mu\text{g/mL}$).⁴ Cells were harvested during log phase and resuspended to 2×10^6 per mL. Eight μL of cell suspension was incubated with each drug concentration in triplicate wells of a 96-well flat-bottomed microtitre plate. Cells cultured in drug-free medium were served as controls for cell survival and wells with only culture medium were used to blank the spectrophotometer. The plates were incubated for 4 days at 37°C in humidified air containing 5% CO_2 . After 4 days, MTT assay was performed according to the manufacturer's instructions. Absorbance was measured at the wavelength of 570 nm in a microtiter plate reader (reference wavelength: 655 nm). The leukaemic cell survival rate was calculated with the equation: (mean OD treated wells/mean OD control wells) $\times 100\%$.

Gene Expression Profiling

The cell lines were cultured with 1.0 $\mu\text{g/mL}$ and 0.4 $\mu\text{g/mL}$ of prednisolone. Cells were harvested after 2 days by Ficoll density gradient centrifugation. RNA was extracted using RNeasy Kit. Total mRNA of 1 μg was reverse transcribed into cDNA and then into cRNA as per manufacturer's recommendation. The Affymetrix HG-U133A chip was used to profile the leukaemia cell lines. Interassay reproducibility was determined in duplicate and was found to be excellent.

Quantitative Real-time RT-PCR

Real-time RT-PCR using the LightCycler was used to determine the level of mRNA for 5 genes that were found to be commonly upregulated among the 3 sensitive cell lines with 0.4 $\mu\text{g/mL}$ of prednisolone. ABL was used as the housekeeping gene and water as a no-template control. The ratio of specific genes copy number against that of ABL was reported. These values were used to correlate with the Affymetrix data.

Results and Discussion

Prednisolone concentration of 0.4 $\mu\text{g/mL}$ was found to be the optimal concentration that separates the 4 cell lines into 3 distinct groups of sensitive, intermediate and resistant. Prednisolone concentration of 1 $\mu\text{g/mL}$ was able to convert the intermediate sensitive cell line to sensitive.

By comparing the changes in gene expression profiles between day 2 treated samples and day 2 untreated controls, we found that there was an exponential increase in the number of significant genes which changed from resistant to intermediate to sensitive cell lines (Table 1).

Analysis of the genes changed, and surprisingly, we found that there were no common genes in cell line Reh (resistant) compared to the genes in the sensitive cell lines. Intriguingly, there was marked overlap in the genes that were significantly changed in the intermediate and sensitive cell lines. Specifically, 5 genes (6 probe sets) were found to be consistently elevated after treatment with 0.4 $\mu\text{g/mL}$ of prednisolone among the 3 sensitive cell lines. This indicates that the

Table 1. Changes in Gene Expression Profile Observed in Cell Lines Incubated with 0.4 $\mu\text{g/mL}$ versus 1.0 $\mu\text{g/mL}$ of Prednisolone

Cell lines		Number of gene changes			
		0.4 $\mu\text{g/mL}$	1.0 $\mu\text{g/mL}$	Common	Percentage (0.4 $\mu\text{g/mL}$)
REH(Resistant)	Increase	4	19	0	0
	Decrease	3	18	0	0
697(Intermediate)	Increase	11	45	9	~82%
	Decrease	3	42	0	0
Sup-B15 (Intermediate)	Increase	187	535	174	~93%
	Decrease	50	505	41	~82%
RS4; 11 (Sensitive)	Increase	391	944	292	75%
	Decrease	270	709	226	84%

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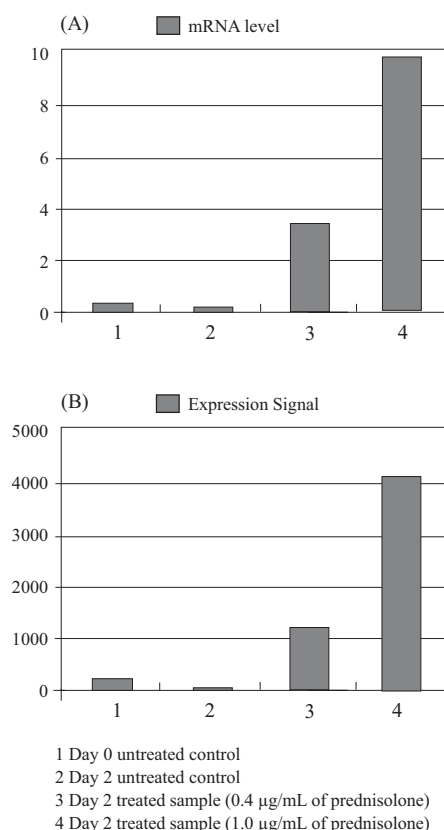


Fig. 1. Expression signal and mRNA level of gene KLF2 in cell line RS4;11 as determined by (A) Affymetrix Genechip HG-U133A and (B) Realtime Quantitative PCR respectively.

gene expressions changed in Reh cell line were random while gene expressions changed in the other 3 cell lines were due to exposure to prednisolone.

The raised genes were confirmed using real-time quantitative PCR. A typical strong correlation between GEP and real-quantitative PCR is shown in Figure 1. In addition, probe sets 204698 at and 33304 at were designed to interrogate different parts of the same gene

ISG20, and both of them turned out to be of the same prognostic value, implying that the changes in gene expressions were not random.

We hereby report that a minimum molecular signature by changes gene expression profiling that is consistently correlated to the response to prednisolone therapy in childhood ALL. This provides the basis for us to study the changes in GEP after 7 days of prednisolone compared to diagnosis in vivo in our current Singapore-Malaysia ALL trial. This will, hopefully, improve our ability to predict the outcome of ALL in children.

Conclusion

In vitro drug sensitivity and gene expression profiling are valuable for disease prognostication in childhood ALL. We hope to extend this finding to patient samples post-therapy with prednisolone in our leukaemia trial.

Acknowledgement

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Young Investigator's Award Finalist

The Effects of Exercise on the Functional Status of the Elderly Persons Living in the Long-term Care Setting of a Psychiatric Hospital

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Introduction

Exercise is an effective way of promoting wellness in people of all ages. It often slows down the process of disability in old age or reverses the decreased mobility that contributes to diseases in old age.¹ The effectiveness of exercise has not been well-evaluated in Asian countries, particularly for elder persons living in long-term psychiatric settings. This study was done to explore the effects of

exercise on the functional status of the elderly persons living in the long-term care setting of a psychiatric hospital.

Materials and Methods

The quasi-experimental design was used for this study. The subjects included in this study were inpatients of the psychiatric hospital aged 65 years and above, who are able to follow simple instructions, have been staying in the ward for more than 6 months,

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are independent in their Activities of Daily Living (ADL) and/or need moderate assistance in their ADL (semi-dependent). Subjects with physical limitations (e.g., fractures), dementia, severe communication difficulties, and who are totally dependent on nursing staff for their ADL were excluded from this study. A sample of 107 elderly who fit the criteria was taken from the psychogeriatric wards of the hospital. Those who participated in the exercise program were grouped into the experimental group, while those who did not participate in the exercise program were grouped into the control group.

Data collection was carried out using a questionnaire, where patients' demographic data, medical history, fall history, exercise involvement, Elderly Mobility Scale (EMS) and Modified Barthel Index (MBI) were recorded. All subjects recruited in the study and who had given their consent for the study were assessed using the EMS and their functional level, using MBI. This was to ascertain the patients' baseline functional and mobility status before the commencement of the study.

A simple, 20-minute exercise program, which consisted of a Range Of Motion (ROM) exercise session and walking exercise of about 60 metres, was introduced to the experimental group for 3 times a week for 3 months. No intervention was introduced to the control group. A post-test recording was taken after the exercise

program and the results were compared with the baseline data. Data analysis was done using the Statistical Package for Social Science version 11.

Results

Of the 107 participants, 17 were males and 90 were females. The mean age of the participants was 73.9 years. All participants suffered from a mental disorder, of which schizophrenia was the commonest followed by depression. Seventy-eight of the participants were independent in their mobility status and 29 of them were semi-dependent. Fifty-two of the participants in the experimental group were more likely to be exercising before (OR = 3.4, 95% CI, 1.4-8.2).

Table 2 shows that the percentage change in MBI and EMS for those in the experimental group was higher than those in the control group. This is significant as it indicates that the simple exercise program did improve the functional status of the elderly persons. The percentage change in the MBI was also higher in those participants who were semi-dependent (11.84%).

Discussion

The study showed that there were significant changes in the MBI scores for those in the experimental group as compared to the control group ($P < 0.001$, Table 2). This is statistically and clinically significant as the Barthel Index is sensitive to change and a score of 1 or 2 may decide the level of dependence of the patient.

There was a significant change in the MBI of those who were semi-dependent as compared to those who were independent (Table 3). This could be due to participants who had a high initial score, and had improved in their scoring where it was beyond what the upper limit score could measure.²

Within the experimental group, there were 52 participants who had exercised before ($P = 0.004$). This group of participants was more likely to exercise because they were interested in the exercise program, enjoyed exercising or liked doing something familiar to them. The kind of exercises they did was mainly walking exercises, which was also part of the exercise program ($P = 0.030$). This could have introduced bias into the study, as participants who liked exercising would have chosen to participate in the study.

Limitations

The findings of this study cannot be generalised towards other populations as the implementation period for the exercise program

Table 1. Demographic Table of the Participants (n = 107)

Characteristics	Frequency	%
Gender:		
Male	17	15.9
Female	90	84.1
Race:		
Chinese	98	91.6
Indian	3	2.8
Malay	3	2.8
Others	3	2.8
Mobility Status:		
Independent	78	72.9
Semi-dependent	29	27.1
Psychiatric problem:		
Schizophrenia	98	91.6
Depression	2	1.9
Others	7	6.5
Medical Problem:		
Yes	46	43
No	61	57

Table 2. Percentage Change in Modified Barthel Index (MBI) and Elderly Mobility Scale (EMS)

		Mean	SD	Minimum	Maximum	Median
Participated in Exercise Programme						
Yes	% change in MBI	-7.77	15.45	-104.35	.00	-2.04
No	% change in MBI	-1.09	5.74	-22.95	12.50	.00
$P < 0.001$ (MWU)						
		Mean	SD	Minimum	Maximum	Median
Participated in Exercise Programme						
Yes	% change in EMS	-6.42	24.72	-180.00	17.39	.00
No	% change in EMS	-.34	19.50	-80.00	61.54	.00
$P = 0.01$ (MWU)						
Change in MBI = pre MBI – post MBI						
Change in EMS = pre EMS – post EMS						

Table 3. Percentage Change in Modified Barthel Index (MBI) in the Mobility Status Groups

		Mean	SD	Minimum	Maximum	Median
Mobility status						
Independent	% change in MBI	-2.57	7.07	-46.77	12.50	.00
Semi-independent	% change in MBI	-11.84	20.62	-104.35	9.68	-6.98

was only 3 months, as some of the patients were transferred from the psychiatric hospital to sheltered homes. Participants were also allocated according to their willingness to participate in the exercise program (experimental group) and those who did not want to participate were grouped into the control group. This could have introduced biases into the study.

Recommendation

Many studies have been conducted to explore the intensity of aerobic activity and the corresponding degree of fitness. However, the exercises were not applicable to those elderly persons who are more debilitated.³ This study was conducted based on a simple exercise program, which is of low-to-moderate intensity. This is best for geriatric patients, where the aim of exercise is to maintain the interest of the elderly persons and avoiding injuries arising from overuse.⁴

Conclusion

The outcome of this study suggests that the effects of exercise on the functional status of the elderly persons living in the long-term care setting of the psychiatric hospital were similar to most studies that were done across countries, on subjects who are mentally capable in the community settings. The introduction of a simple exercise program will help the elderly persons in the psychiatric hospital to stay active instead of sitting around with nothing to occupy them in the day space of the hospital. Besides, the benefits

exercise has on the individual may mean lesser burden on the caregivers, and the time and energy channelled to those who need more nursing care.

Acknowledgement

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Young Investigator's Award Finalist

The Impact of Microanastomosis of the Intramuscular Nerve Branch on the Healing of a Completely Lacerated Skeletal Muscle: A Histopathological Analysis

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Introduction

A completely lacerated skeletal muscle that is repaired by suturing the cut muscle ends often results in an incomplete recovery. Garret et al¹ have reported histological abnormalities, characterised by muscle fibre atrophy, increased degree of fibrosis, as well as denervation of the segment distal to the laceration, which has been isolated from a nerve supply when the muscle was cut. Although various approaches have been attempted to enhance muscle regeneration and to reduce fibrosis formation, muscle function of lacerated muscles after muscle repair generally do not recover fully.² The surgical repair of the severed intramuscular nerve branches, concomitant to skeletal muscle lacerations, is perhaps ignored as it requires microsurgical skills and expertise.

We hypothesise that a poor neuromuscular reinnervation might, in part, account for the incomplete healing of lacerated muscle repaired by epimysial suturing alone, and that the integrity of the intramuscular nerve might play a role in the recovery process. This study aims to investigate the long-term morphologic changes in a completely

lacerated muscle that is repaired with micro-anastomosis of the main intramuscular (IM) nerve, followed by epimysial suturing of the cut muscle ends.

Materials and Methods

The medial gastrocnemius (MG) in adult NZ White rabbits were used as the model in this study (n = 20). The MG of 1 limb was lacerated completely at the proximal quarter of the muscle belly, just distal to the motor point of the branch from the tibial nerve. The muscle was immediately repaired by epimysial suturing the cut muscle ends together. Two different muscle repair options were used; in 1 group only a muscle repair was done without the IM nerve repaired (non-NR group) and in the other the microanastomosis of the main IM nerve branch, before the muscle repair was done [i.e., the Nerve-Repair (NR) group]. A third group was used as a positive control, where the IM nerve was identified and left intact when the muscle was lacerated, and then the muscle belly was repaired [i.e., the Nerve-Preserved (NP) group]. The non-operated contra-lateral MG in each group serves as normal controls (NC). Removed muscles

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were snap-frozen and transversely sectioned for histochemistry and immunohistochemistry (IHC) staining. The non-NR and NR groups were analysed at the fourth and seventh month postoperatively, while the positive controls, NP group were evaluated only at 7th month after repair. Only the segment distal to the laceration site was assessed in this study.

Muscle regeneration was quantified through IHC for neonatal myosin heavy chain (MHCn), double-stained for intermediate filament, vimentin and desmin. Vimentin was used as a marker for fibrosis. This was further confirmed with Masson's-modified trichrome. Acetyl cholinesterase (AChE) staining for motor endplate was employed to evaluate the neuromuscular innervation. AChE staining was also coupled with neonatal myosin heavy chain (MHCn) immunostaining to identify the relationship between them. The cross-sectional area (CSA) of total myofibres and regenerating myofibres, area of fibrosis and proportion of muscle types, were quantified and analysed using a computerised imaging system linked to the microscopy.

The average and standard deviation of all data were compared among the different groups using one-way ANOVA for statistical analysis. Statistical significance was defined as $P < 0.05$.

Results

Muscle Regeneration (Fig. 1)

After 4 months, the CSAs of the total myofibres in the NR group were noted to be significantly higher than the non-NR group. In addition, the NR group had a higher number of regenerating myofibres with larger CSAs than the non-NR group. After 7 months, the

number of regenerating myofibres in the NR group decreased significantly, compared to the non-NR group. The CSAs of total myofibres and regenerating fibres in the NR group, when compared to the positive control, the NP group, at 7 months, were however smaller and fewer in number. In the NP group, the CSA of total and regenerating fibres did not statistically differ from collateral normal controls (NC).

Muscle Fibrosis (Fig. 2)

Vimentin and Masson's trichrome staining showed no difference in area of fibrosis between non-NR and NR groups at the fourth month. Fibrosis formation in both groups, however, was significantly increased, compared with contra-lateral control. In contrast, the NR group showed significantly decreased fibrosis formation in comparison with non-NR group at 7 months. There was no difference in area of fibrosis between the NR group and the NP group, and between the NP group and contra-lateral normal controls, at 7 months.

Muscle Innervation (Fig. 1)

Poorly organised, disconnected, randomly distributed AChE-positive motor end-plate spots, featured as multiple and polyneuronal reinnervation suggestive of nerve terminal spouting, was observed in the non-NR group, at both time-points. In contrast, the NR group demonstrated a well-organised, interconnected band of AChE-positive motor endplate spots that was similar in pattern to the positive (NP) and normal (NC) controls, but it demonstrated a less dense staining than those of the controls, at the fourth month and the seventh month. No difference was found between the NP and NC controls at 7 months.

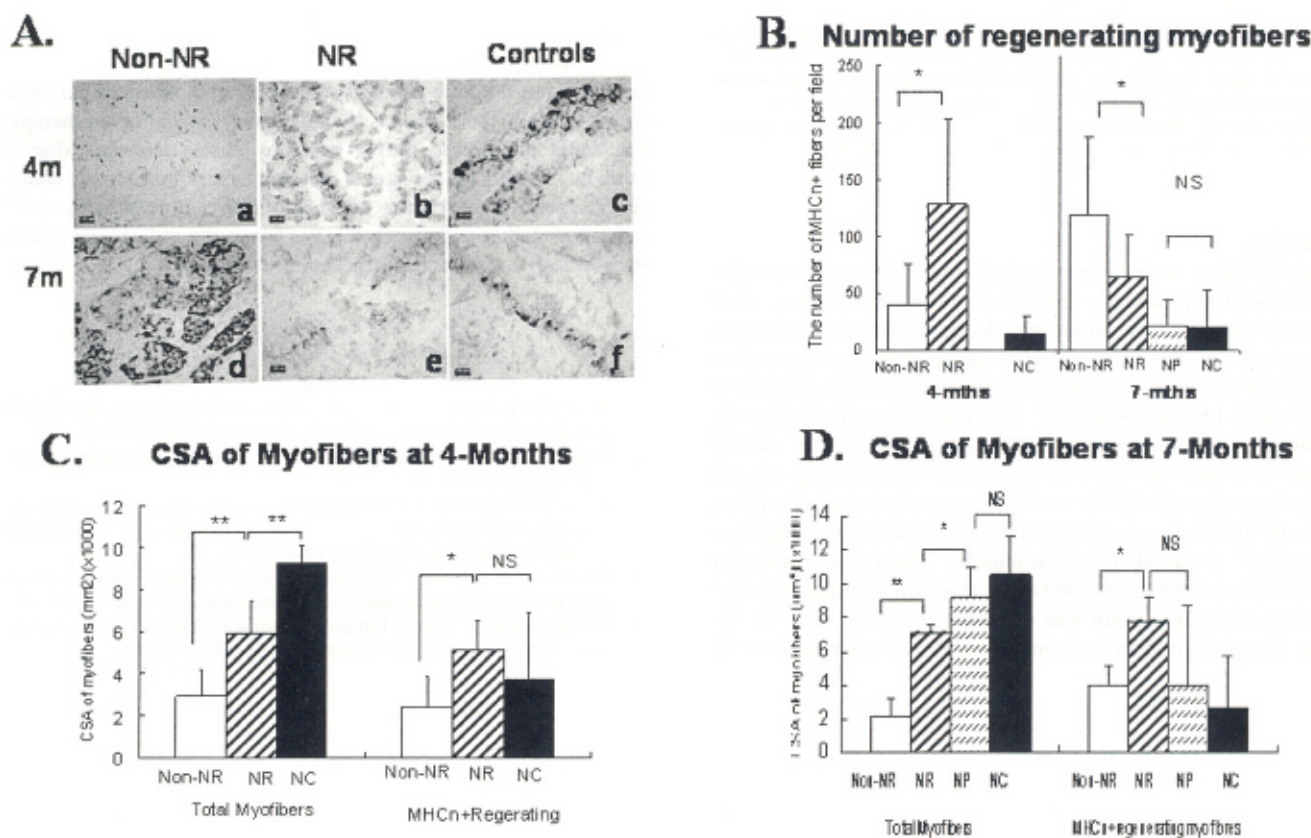


Fig. 1. Quantification of total and regenerating myofibre size and regenerating myofibre number in distal segment at 4 months (a-b) and 7 months after repair (d-f). (A): AChE stains (dark blue color) coupled with MHCn (brown) immunostaining. (a,d): Non-nerve repair (Non-NR); (b,e): Nerve-repair (NR); (c): Contra-lateral normal control (NC); (d): Positive control, nerve preserved (NP). * $P < 0.05$; ** $P < 0.01$; NS: not significant. (B): The number of regenerating myofibres per field at 4 months and 7 months (C) CSA of total and regenerating myofibres at 4 months (D) CSA of total and regenerating myofibres at 7 months.

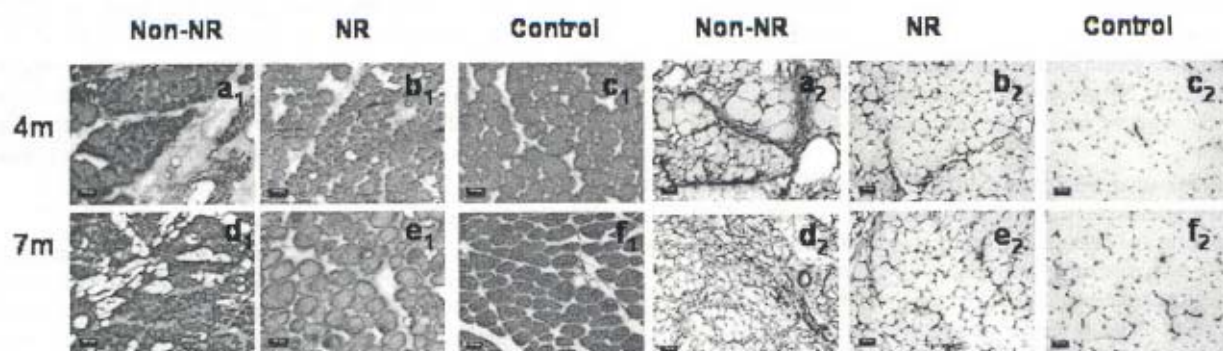
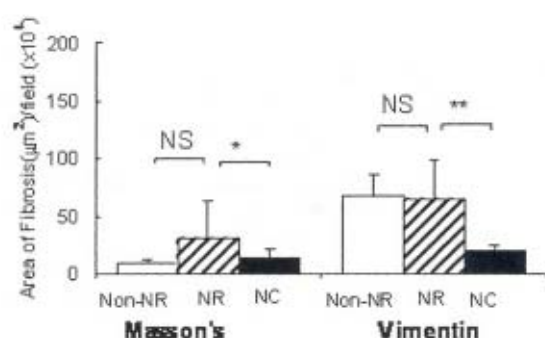
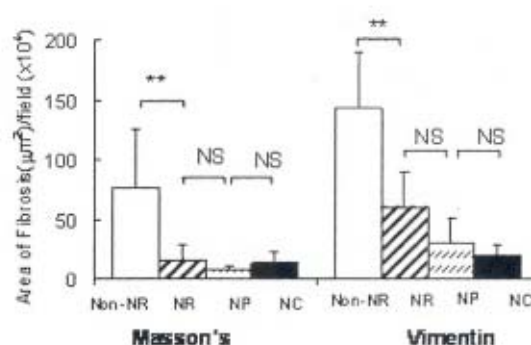
A.**B.****Area of Fibrosis at 4-Months****Area of Fibrosis at 7-Months**

Fig. 2. Quantitative histological analysis of fibrosis formation in distal segment detected by Masson's Trichrome (a₁-f₁) and Vimentin (a₂-f₂) immunostaining, 4 months and 7 months after repair. **Top panel (A):** Masson's Trichrome (collagenous area: blue) and Vimentin immunostaining (fibrosis area: brown); **Bottom panel (B):** area of fibrosis per field. Note: Non-NR: non-nerve repair; (NR): nerve repair; NC: contra-lateral normal control (c₁, c₂) and NP: nerve preserved (f₁, f₂). *P < 0.05; **P < 0.01; NS: not significant

Discussion

The NR group, where intramuscular nerve branch was repaired, demonstrated substantially improved muscle morphology with enhanced muscle regeneration and decreased fibrosis formation, especially at 7 months. Recent studies suggest that innervation plays an essential role in promoting regenerating muscle fibre maturation and blocking fibrosis formation^{3,4}. This was also confirmed by our findings of appropriate innervation present in both the NR and NP groups, in contrast to the poor innervation noted in the non-NR group. Clinically, the findings from this study provide a rationale for repair the intramuscular nerve branch which has further improved the recovery of lacerated skeletal muscles. Nevertheless, a slower morphologic recovery correlated to the less dense staining of motor endplates in the NR group was noted when compared to the NP group. This is more likely the result of a slower re-innervation of the

muscle given the time gap difference for nerve regeneration after repair, between the 2 groups. In this context, an additional-therapy that could promise to further improve muscle recovery after a laceration would be the use of growth factors that would either promote nerve or muscle regeneration, or reduce fibrosis formation,⁵ and future studies will be necessary to assess the earlier time-points so as to fully understand the mechanisms involved.

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Between the Rod and Reason: A Study on Asian Parental Disciplinary Methods and Child Emotional/Behavioural Outcomes

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Introduction

In contemporary, Western-based, literature, Asian parenting is frequently described as authoritarian.¹ Authoritarian parents value control and expect children to conform to a standard set of conduct and punish them for violating it.² Hence, it has been a common notion that authoritarian, and therefore, Asian parents are in favor of physical punishment to gain compliance.

Research on the effect of physical punishment on child outcomes yielded mix findings. It has been reported that frequent physical punishment is associated with negative child outcomes, such as emotional disorders, while child compliance as a result of punishment has been regarded as a desirable outcome.³

Most of these studies have been conducted with Western populations. From the classic writing of Whiting and Whiting⁴ on the *Children of Six Cultures*, it is evident that child-rearing practices and child development vary across cultures. Hence, this calls for a need for more research on the nature of Asian parenting and child outcomes in the Asian context, which has been scanty.

In the present study, we sought to understand Asian parental disciplinary practices and their associations with child behaviours. We first examined the most frequent form of disciplinary practice used by parents of children seen at a child mental health facility in Singapore; disciplinary practices were operationalised as: Reasoning with the child, caning the child or a combination of both practices. Caning was taken to be a form of punitive parenting practice in this context. The association between these disciplinary practices and child emotional/behavioural outcomes was also investigated.

Materials and Methods

Participants were 230 Singapore parents (63 males and 167 females; aged 23 to 52 years old, M = 40.7 years; SD = 4.4 years) whose children (173 boys and 57 girls; aged 4 to 12 years old, M = 8.7 years; SD = 1.8 years) were attending the Child Guidance Clinic. Parents reported how they would react when their child has done something wrong, and scored their child's behaviour in the Child Behaviour Checklist (CBCL/4-18).⁵ The CBCL/4-18 comprises of eight syndrome scores (namely, Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behaviour and Aggressive Behavior), 2 syndrome group scores (namely, Internalising/Emotional-based problems and Externalising/Behavioural-based problems) and a total score.

Results

From Figure 1, it could be seen that most parents do not involve caning as a form of disciplinary practice. 68.3% of the parents would employ reasoning, while only 31.7% of the parents would involve caning, when disciplining their children.

One-way ANOVAs showed that (1) compared to parents who used caning as a disciplinary method (with or without reasoning), parents who do not cane their children at all reported significantly the least

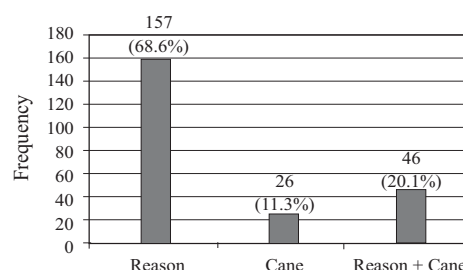


Fig. 1. Frequency of disciplinary practices.

Table 1. Mean Scores of CBCL Across Types of Disciplinary Practices

	Disciplinary practices			F	P
	Reason	Cane	Reason + Cane		
Withdrawn	3.94 (3.43)	4.92 (3.62)	3.38 (3.08)	1.74	ns
Somatic complaints	2.41 (2.80)	3.23 (2.79)	2.09 (2.65)	1.45	ns
Anxious/depressed	5.64 (5.15)	7.85 (5.99)	5.17 (4.47)	2.51	ns
Social problems	4.29 (3.27)	5.50 (3.06)	4.49 (2.64)	1.66	ns
Thought problems	2.37 (2.52)	2.77 (2.44)	2.36 (2.01)	0.32	ns
Attention problems	7.89 (4.69)	10.31 (3.42)	9.26 (3.72)	4.43	<0.05
Delinquent behaviour	2.48 (2.49)	4.15 (2.94)	3.47 (2.40)	6.53	<0.01
Aggressive behaviour	10.39 (7.42)	14.81 (7.87)	13.62 (8.09)	5.95	<0.01
Internalising problems	11.56 (9.23)	15.58 (10.06)	10.30 (7.66)	2.98	ns
Externalising problems	12.87 (9.48)	18.96 (10.24)	17.09 (9.81)	6.71	<0.01
Total problems	44.17 (27.35)	59.27 (29.08)	49.06 (24.21)	3.69	<0.05

ns: not significant

* Values in parentheses denote standard deviations

externalizing problems (including attention problems, delinquent behaviour, aggressive behaviour) in their children, and (2) compared to parents who used either reasoning or caning solely as their respective disciplinary method, parents who used a combination of reasoning and caning reported the least internalizing problems (including withdrawn syndrome, somatic complaints, anxiety/depression) in their children, though the findings did not reach statistical significance (Table 1).

Discussion

Contrary to prevailing belief, caning was not the most commonly

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used form of disciplinary practice engaged by Asian parents; reasoning was found to be used most frequently in the present study, suggesting that Asian parents might not be as punitive as suggested by previous research on Asian parenting (with punitive being strictly defined as caning in the present context).

Past research showed that physical punishment tends to predict higher rates of externalizing problems.⁶ This pattern of association was observed in the present study as well. This could be explained using the learning theory, in which children learned from the parents' caning that violence is a solution to interpersonal conflict. Over time, these children become aggressive too.

Interestingly, our finding further revealed that reasoning alone does not seem to be the best disciplinary practice across context, as parents who used a combination of reasoning and caning reported the least internalizing problems in their children, although this result did not reach statistical significance.

Conclusions

Our data shed some light on the effects of physical punishment on child behaviour and mental health in the Singaporean context, but this remains a complex issue. A significant lesson learnt is that different parental disciplinary practices might be related to different aspects of child well-being, and a clinical implication is that depending on the type of problems displayed by children, parental disciplinary

methods must be adjusted accordingly.

The present study contained some limitations. By using a clinical sample, external validity is low. Furthermore, referral bias might have affected the informant ratings. In future studies, a non-referred sample like a community sample should be used. In addition, it may be useful to have a child self-rating form, which we did not have in the present study.

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Best Oral Presentation Award Finalist – Allied Health/Health Sciences/Paramedical Disciplines

Anthropometric Indices as Screening Tools for Cardiovascular Risk Factors in Singaporeans: Receiver Operating Characteristic Curves Analysis

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Introduction

Obesity is an independent risk factor for cardiovascular diseases (CVD), and is strongly associated with risk factors like hypertension, dyslipidaemia and diabetes. From the public health perspective, it is important to define cut-off values for obesity indices such as body mass index (BMI), waist to hip ratio (WHR), and waist to stature ratio (WSR) to achieve effective screening. The World Health Organization (WHO) has recommended WHRs of 1.0 and 0.85 as obesity cut-off points for males and females, and BMI of $\geq 25\text{kg/m}^2$ and $\geq 30\text{kg/m}^2$, for overweight and obesity respectively.¹ In Asia, many studies have indicated the need to revise these values.²⁻⁶ In Singapore, Deurenberg-Yap et al⁵ demonstrated that for the same BMI, Singaporean adults displayed higher body fat percentages than Europeans. In another study, CVD risks were found to increase at lower BMI and WHR levels.⁶ In these studies, however, no specific BMI and WHR cut-off values were proposed. Moreover, no cut-off values for WSR were recommended by the WHO, although it has been suggested that WSR may be the most appropriate screening tool.^{2,3} Therefore, the purpose of this study was to delineate cut-off points of BMI, WHR, WSR in Singaporeans based on 3 CVD risk factors (hypertension, diabetes and dyslipidaemia).

Materials and Methods

The subjects were 730 hospital employees (61% Chinese, 27% Malays and 12% Indians), who underwent health screening after an overnight fast. Waist and hip circumferences (measured to nearest 0.1cm) were measured midway between the last rib and iliac crest, and over the greater trochanters, respectively. WHR and WSR were then calculated, while BMI was calculated as weight (kg) divided by height squared (m^2). Blood pressure (BP) was measured using a digital sphygmomanometer. Serum levels of total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol triglycerides (TG), and blood glucose concentration were measured using enzymatic methods.

Hypertension was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg. Dyslipidaemia was defined as TC ≥ 6.2 mmol/L, and/or elevated TC/HDL ratio ≥ 4.4 , and/or TG ≥ 1.4 mmol/L. Type 2 diabetes mellitus was defined as fasting blood glucose ≥ 7.0 mmol/L. "At risk" was defined as having at least one risk factor.

Statistical Analysis

Using SPSS for Windows (v9.01), Receiver Operating Characteristic (ROC) curves were plotted for each index, and the optimal cut-off value was one that yielded the largest sum of sensitivity and specificity. Area

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Table 1. Anthropometric Indices and Cardiovascular Risk Factors by Gender and Ethnic Group (mean $\pm$ SD)

Variables	Males (n = 164)			Females (n = 566)		
	Chinese (n = 104)	Malay (n = 42)	Indian (n = 18)	Chinese (n = 337)	Malay (n = 157)	Indian (n = 72)
Age (y)	40.7 $\pm$ 12.0	42.2 $\pm$ 9.7	39.2 $\pm$ 11.1	39.0 $\pm$ 12.0	35.2 $\pm$ 12.0	39.4 $\pm$ 11.4
Weight (kg)	69 $\pm$ 12.8	70 $\pm$ 12.4	77 $\pm$ 17.9*	55.1 $\pm$ 8.9	60 $\pm$ 12.4*	63 $\pm$ 13.8*
Height (cm)	168.4 $\pm$ 6.9	165.9 $\pm$ 6.5	167.7 $\pm$ 9.9	157 $\pm$ 6.3	155 $\pm$ 6.2	155 $\pm$ 5.6
Waist (cm)	84.1 $\pm$ 10.1	84.6 $\pm$ 10.4	91.9 $\pm$ 13.3*	72.0 $\pm$ 8.5	76.0 $\pm$ 5.7*	81.0 $\pm$ 13.0*
Hip (cm)	96.8 $\pm$ 7.7	96.5 $\pm$ 7.6	101.9 $\pm$ 11.1*	93.2 $\pm$ 8.0	97.1 $\pm$ 9.9*	100.7 $\pm$ 11.6*
BMI (kg/m ²)	24.3 $\pm$ 4.2	25.4 $\pm$ 4.2	27.5 $\pm$ 6.8*	22.2 $\pm$ 5.5	24.8 $\pm$ 5.2*	26.2 $\pm$ 5.4*
WHR	0.87 $\pm$ 0.05	0.87 $\pm$ 0.05	0.90 $\pm$ 0.05*	0.77 $\pm$ 0.05	0.78 $\pm$ 0.06*	0.80 $\pm$ 0.07*
WSR	0.50 $\pm$ 0.06	0.51 $\pm$ 0.07	0.55 $\pm$ 0.08*	0.46 $\pm$ 0.06	0.49 $\pm$ 0.07*	0.52 $\pm$ 0.08*
TC (mmol/L)	5.5 $\pm$ 0.93	6.1 $\pm$ 1.1*	5.7 $\pm$ 1.4	5.5 $\pm$ 1.0	5.4 $\pm$ 0.9	5.6 $\pm$ 0.9
HDL (mmol/L)	1.5 $\pm$ 0.33	1.3 $\pm$ 0.31*	1.2 $\pm$ 0.30*	1.9 $\pm$ 0.43	1.7 $\pm$ 0.39*	1.5 $\pm$ 0.42*
LDL (mmol/L)	3.3 $\pm$ 1.0	3.8 $\pm$ 1.1*	3.5 $\pm$ 1.5	3.2 $\pm$ 0.9	3.2 $\pm$ 0.9	3.6 $\pm$ 0.8*
TG (mmol/L)	1.4 $\pm$ 0.89	2.0 $\pm$ 1.5*	2.0 $\pm$ 2.5*	1.1 $\pm$ 0.74	1.1 $\pm$ 0.72	1.2 $\pm$ 0.56*
TC/HDL	3.9 $\pm$ 1.0	4.8 $\pm$ 1.5*	5.0 $\pm$ 2.0*	3.0 $\pm$ 0.8	3.4 $\pm$ 0.9*	3.9 $\pm$ 1.2*
SBP (mmHg)	126.9 $\pm$ 14.4	126.1 $\pm$ 14.8	122.7 $\pm$ 12.1	115.3 $\pm$ 14.8	117.0 $\pm$ 15.1	117.7 $\pm$ 15.5
DBP (mmHg)	80.1 $\pm$ 10.3	82.0 $\pm$ 11.2	77.7 $\pm$ 9.5	74.1 $\pm$ 9.3	75.0 $\pm$ 9.3	75.7 $\pm$ 9.7
Glucose (mmol/L)	5.4 $\pm$ 0.95	5.9 $\pm$ 2.3	5.1 $\pm$ 0.49	5.1 $\pm$ 0.73	5.4 $\pm$ 2.1*	5.6 $\pm$ 1.6*
Hypertension (%)	24.0	19.0	16.7	10.2	10.2	11.1
Dyslipidaemia (%)	49.0	81.0*	66.7*	32.3	35.7	45.8
Diabetes (%)	4.8	11.9	0	3.3	6.4	9.7
Risk (%)	56.7	81.0*	66.7*	37.4	39.5	55.6*

BMI: body mass index; DBP: diastolic blood pressure; glucose, fasting plasma glucose; HDL: high-density lipoprotein cholesterol; Hip: hip circumference; LDL: low-density lipoprotein cholesterol; Risk: at least one CVD risk factor (hypertension or diabetes or dyslipidaemia); SBP: systolic blood pressure; TC: total cholesterol; TG: fasting triglycerides; Waist: waist circumference; WHR: waist-to-hip ratio; WSR: waist-to-stature ratio

*Significant differences in means between ethnic groups (age-adjusted for all variables, Chinese as reference category).

Table 2. Optimal Cut-off Values, Sensitivity, Specificity, and Area Under Curve of Various Anthropometric Indices to Predict Hypertension, Type II Diabetes Mellitus, Dyslipidaemia Based on ROC Analysis

Indices	CVD Risk Factor	Males (n = 164)				Females (n = 566)			
		Cut-off	Sens.(%)	Spec.(%)	AUC(95%CI)	Cut-off	Sens.(%)	Spec.(%)	AUC(95%CI)
BMI (kg/m ²)	Hypertension	24.8	64.5	63.5	0.67 (0.57-0.76)	23.4	83.3	61.3	0.76 (0.70-0.80)
	Dyslipidaemia	24.2	68.5	61.7	0.69 (0.60-0.77)	23.9	55.0	71.7	0.66 (0.61-0.71)
	Diabetes	24.1	90.0	44.5	0.67 (0.50-0.83)	23.2	96.4	57.4	0.80 (0.74-0.86)
	Risk	24.2	67.4	64.3	0.72 (0.63-0.80)	23.6	59.6	73.1	0.69 (0.65-0.74)
WHR	Hypertension	0.89	69.4	64.8	0.67 (0.56-0.77)	0.80	65.0	74.3	0.74 (0.68-0.80)
	Dyslipidaemia	0.86	73.2	53.7	0.64 (0.55-0.73)	0.80	54.0	74.7	0.67 (0.62-0.71)
	Diabetes	0.85	90.0	30.5	0.69 (0.55-0.84)	0.82	71.4	79.4	0.81 (0.74-0.89)
	Risk	0.86	72.2	57.1	0.67 (0.58-0.76)	0.80	54.4	77.5	0.69 (0.65-0.74)
WSR	Hypertension	0.48	91.7	39.1	0.67 (0.58-0.77)	0.51	68.3	77.9	0.79 (0.73-0.84)
	Dyslipidaemia	0.50	63.9	68.7	0.69 (0.60-0.77)	0.48	62.6	71.7	0.70 (0.65-0.75)
	Diabetes	0.50	80.0	48.1	0.61 (0.44-0.79)	0.50	92.8	68.4	0.84 (0.78-0.89)
	Risk	0.48	80.5	57.1	0.72 (0.63-0.80)	0.48	64.5	76.0	0.73 (0.69-0.78)

AUC: area under curves; BMI: body mass index; Risk: at least one CVD risk factor (hypertension or diabetes or dyslipidaemia); Sens: Sensitivity; Spec: Specificity; WHR: waist-to-hip ratio; WSR: waist-to-stature ratio

under the curve (AUC) and forward multiple regression analysis were applied to determine the best index. Adjustment was made for age, ethnicity, smoking status, and physical activity levels. Alpha level was set at 0.05 (2-tailed).

Results

The subjects' anthropometric and metabolic characteristics are shown in Table 1. The cut-off values of the indices are listed in Table 2. As an overall estimation, the cut-off values of BMI, WHR and WSR in predicting at least one risk factor were 24.2 kg/m², 0.86 and 0.48 in men, and 23.6 kg/m², 0.80 and 0.48 respectively in women. Including ties, the AUC of WSR was highest for all 3 risk factors in females (0.79 for hypertension, 0.70 for dyslipidaemia, 0.84 for diabetes mellitus), and 2 risk factors in males (0.67 for hypertension, 0.69 dyslipidaemia). As an overall estimation, WSR had the largest AUC for the presence of at least one CVD risk factor in both males and females (0.72 and 0.73 respectively).

All 3 indices were significantly inter-correlated ($P < 0.001$). In particular, strong and positive correlations were found between

WSR and the other 2 indices ($r = 0.89$ and 0.79 with BMI and WHR respectively in males, 0.85 with BMI in females). Results of regression analysis revealed that WSR was independently associated with all risk factors in females, and dyslipidaemia in males, while BMI was associated with hypertension and diabetes in males.

Discussion

Our study indicated that the cut-off values of BMI and WHR are lower than those recommended by the WHO¹. Applying the WHO's BMI cut-off points for overweight and obesity, the specificity of BMI in predicting risk factors was more than 80%, but the sensitivity was less than 50%. The WHO recommends WHRs of 1.0 and 0.85 for males and females respectively, resulting in a high specificity (>90%) and a low attendant sensitivity (<15%) in our study. Taken together, these results further indicate that the cut-off values by WHO are inappropriately high for Singaporeans.

Few studies have examined the association between CVD risk factors and obesity indices at various cut-off values in Singaporeans. In Deurenberg-Yap's study,⁶ it was found that at low categories of BMI (22-24 kg/m²) and WHR (0.80-0.85 for women, 0.90-0.95 for

men), the odds ratios (ranging from 1.97 to 4.38) for having at least one risk factor were significantly higher compared to the reference category. Using ROC analysis, our results are consistent with that of Deurenberg-Yap's,⁶ and extend their findings to include specific BMI cut-off values at 24.2 and 23.6 kg/m², WHR cut-off values at 0.86 and 0.80, and WSR cut-off values of 0.48 for men and women respectively.

In relation to predicting at least one risk factor, WSR was the only index with AUC greater than 0.70 (0.72 and 0.73 in men and women, respectively). Using regression analysis, WSR was selected as the main predictor for both sexes. Moreover, the cut-off value of 0.48 was the same for both genders. Taken together, these results indicate that WSR may be the most appropriate screening tool.

Comparing our results with that of other studies performed in Asia, the cut-off values in relation to each risk factor were comparable with those obtained in Japanese men,⁴ Hong Kong Chinese² and Taiwanese.³ However, our results were apparently higher than values obtained from Japanese women. In our study and that of others,^{2,3} the cut-off point was one that yielded the largest sum of sensitivity and specificity. In contrast, Ito et al⁴ determined cut-off values at a point where sensitivity equated to specificity. Notwithstanding the methodological differences, we believe our study has provided convergent evidence to support recent revisions of obesity indices to represent over-weight for Asians.

Conclusion

The cut-off points of anthropometric indices in Singaporean

adults were lower than the WHO criteria, but were in agreement with those reported for Asians.²⁻⁴ The WSR may be the best screening tool, and such a conclusion awaits further verification in prospective or longitudinal epidemiological studies on morbidity and mortality.

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Best Oral Presentation Award Finalist – Allied Health/Health Sciences/Paramedical Disciplines

CC3/TIP30 Expression was Strongly Associated with HER-2/NEU Status in Breast Cancer

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Introduction

Cancer metastasis is a complicated process in tumour development. It involves up- and down-regulation of genes in malignant tumour cells. A human gene, *CC3*, has been identified as a novel metastasis suppressor gene of variant small-cell lung carcinoma (v-SCLC).¹ This gene is identical to the transcription cofactor, *TIP30*, which can bind to the human immunodeficiency virus (HIV) Tat protein and initiates Tat-activated transcription.² Overexpression of the *CC3/TIP30* gene resulted in suppression of the metastatic potential of v-SCLC cells in SCID-hu mice and of mouse melanoma. *CC3/TIP30* also inhibited angiogenic properties of tumour cells by inducing changes in the RNA level of angiogenic modulators.³ This led to the proposal that *CC3/TIP30* functions as a metastasis suppressor gene, linking the control of apoptosis to metastasis. However, its expression profile in clinical specimens, especially in breast cancer, has not been reported.

In this study, we investigated the differential expression of *CC3/TIP30* in breast tumour and matched normal tissues. We observed that overexpression of *CC3/TIP30* was mostly found in HER-2/*neu* positive breast carcinoma. Our results may indicate the potential involvement of *CC3/TIP30* in the enhanced breast tumour differentiation, proliferation and invasion caused by the HER-2/*neu* proto-oncogene.

Materials and Methods

Total RNA was extracted from 43 pairs of frozen breast tumour/normal tissues with previously characterized HER-2/*neu* status.⁴ The relative levels of *CC3/TIP30* and β -actin were determined by semi-quantitative RT-PCR and reverse-phase protein array. The latter was performed to ascertain whether *CC3/TIP30* was differentially expressed at protein level in the two subtypes of breast cancer. Immunohistochemical staining of sections from a breast cancer tissue microarray containing 97 breast tumours and matched normal

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tissues⁵ were carried out to study the expression of the *CC3/TIP30* protein and its potential association with various clinicopathological parameters. Full length *CC3/TIP30* cDNA was amplified and sequenced for mutation detection. SPSS v12.0 software and Fisher's exact test were used for statistical analysis. A value of $P < 0.05$ was considered statistically significant.

Results

RT-PCR. *CC3/TIP30* was successfully amplified in 39 pairs of tissues and its relative expression levels were normalized with β -actin. The mean value of relative expression in these 43 cases was defined as 0.17 (95%CI: 0.11-0.24) for all the matched normal tissues (N = 39), 0.57 (95%CI: 0.41-0.73) for HER-2/*neu* negative tumour tissues (N = 29) and 1.08 (95%CI: 0.91-1.25) for the HER-2/*neu* positive tumour tissues (N = 10). In Figure 1 (panel I), the overall relative expression level of *CC3/TIP30* in the cohort of HER-2/*neu* positive tumours was significantly higher than that in the HER-2/*neu* negative tumours and normal tissues ($P = 0.023$).

Reverse-phase protein array. Total proteins from 10 HER-2/*neu* positive and 20 -negative tumour tissues and 20 normal tissues were spotted onto the PVDF membrane and the relative expression of

CC3/TIP30 to β -actin were analyzed. As shown in Figure 1 (panel II), *CC3/TIP30* was significantly overexpressed in HER-2/*neu* positive breast tumours ($P = 0.016$).

Immunohistochemical staining of breast tissue microarray. No staining was detected in normal tissues. In the tumour tissues, *CC3/TIP30* was stained only in cytoplasm (Fig. 2). Only 68 cases with *CC3/TIP30* staining status were available for analysis. In the 17 HER-2/*neu* positive tumours, 7 cases were stained strongly, 5 moderately and 5 weakly, whereas in 51 HER-2/*neu* negative tumours, 7 cases were strongly stained, 16 moderately and 28 weakly and/or no staining ($P = 0.027$). There were no significant associations with ER, PR, lymph node and tumour grade and size.

Full length cDNA sequencing. To understand whether the abnormal expression of *CC3/TIP30* was caused by mutations, full length cDNA from 5 cases with high expression and 5 cases with low expression level of *CC3/TIP30* and 8 normal cases were amplified and sequenced. Four single nucleotide polymorphisms in the *CC3* coding region (listed in the SNP database) were excluded when comparing the sequence with *CC3/TIP30* cDNA. Of these 18 cases, no insertion, deletion and single bp mutations which cause amino acid substitutions were found in the coding region.

Discussion

In this study, we observed that *CC3/TIP30* is mostly overexpressed in HER-2/*neu* positive breast carcinomas at both transcription and translation levels. To our knowledge, this is the first finding that shows *CC3/TIP30* expression associated with HER-2/*neu* status in breast cancer. In contrast to published results, our findings may implicate that in breast carcinoma, *CC3/TIP30* has different functions in primary and metastatic tumour cells or that its functions could be tumour-dependent or that its cellular functions could depend on the balance between the regulated pro- and anti-apoptotic molecules.

Our present data demonstrated a possible association of *CC3/TIP30* with the HER-2/*neu* oncogene or its downstream signal pathway. HER-2/*neu* is an established adverse prognostic factor in breast cancer and is strongly associated with a more aggressive phenotype HER-2/*neu* activation also initiates signaling cascades

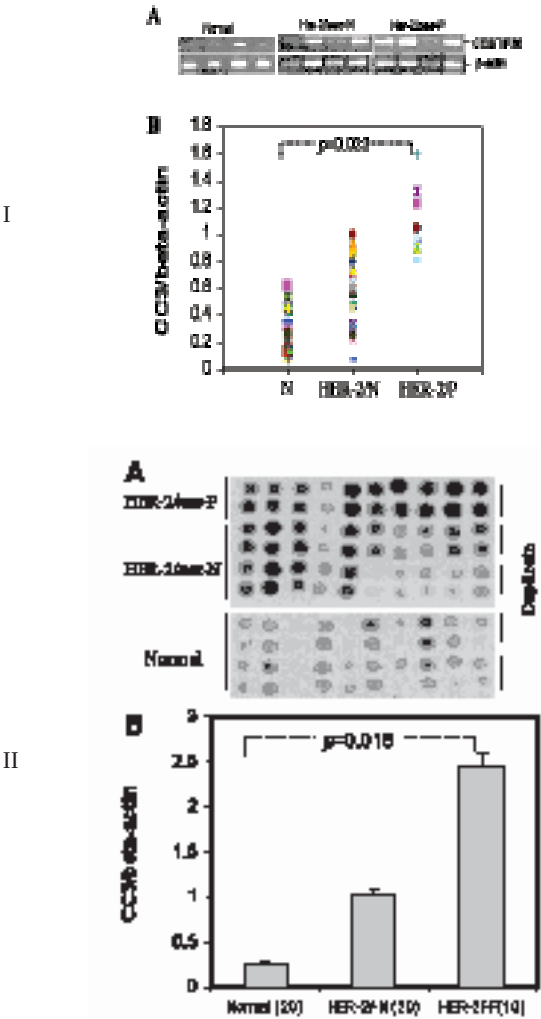


Fig. 1. Reverse transcription (RT)-PCR (Panel I) and Reverse phase protein array (Panel II) of *CC3/TIP30*. (Panel I) A representative amplification of *CC3/TIP30* (top) and expression level of *CC3/TIP30* in normal and tumour tissues. (Panel II) Signal detection of *CC3* protein (top) and relative to β -actin.

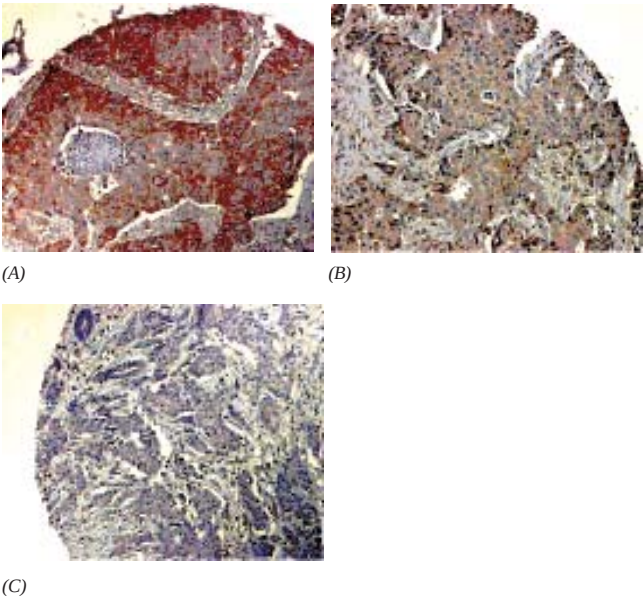


Fig. 2. Immunohistochemistry staining of *CC3/TIP30* in breast cancer tissue microarray showing strong staining (A), moderate staining (B) and negative staining (C) respectively. (Original magnification x200)

including the MAPK and PI3K/AKT pathways that are essential for cell proliferation and differentiation. The finding that *CC3* was overexpressed in the TGF- β 1-treated T84 cells implied the possible function of *CC3* in cell differentiation. However, the biological function of *CC3/TIP30* and its connection with HER-2/*neu*-related signal pathways in breast cancer remain elusive.

Based on the immunohistochemical staining, we observed that *CC3/TIP30* was present only in the cytoplasm. We did not find any cases showing nuclear staining. To understand whether the cytoplasmic localization of *CC3* was due to the site mutation at codon position 106 (R to H) as reported [6], we amplified and sequenced the full cDNA of *CC3/TIP30*. No mutations, insertions and deletions were found in these limited specimens, hence more breast carcinoma cases may need to be screened.

Conclusion

Our data showed that *CC3/TIP30* expression is strongly associated with HER-2/*neu* oncogene in breast cancer. Cytoplasmic localization of *CC3/TIP30* may indicate the functional variance of *CC3/TIP30* in breast cancer with SCLC and other tumour types. The identification

of differentially expressed gene and/or protein profiles initiated by *CC3/TIP30* in breast cancer cell lines will unravel, at least in part, the molecular events that may provide novel cross-talk between the signal networks and contribute to a better understanding of *CC3/TIP30*-mediated cell behavior. The association between increased expression of *CC3/TIP30* and HER-2/*neu* positivity presents an interesting paradox.

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Best Oral Presentation Award Finalist – General Practice

A KAP Survey of Evidence-Based Medicine and Clinical Practice Guidelines Among Primary Care Doctors in Singapore

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Introduction

Primary healthcare doctors working in the National Healthcare Group Polyclinics and also private General Practitioners who are affiliated to the National Healthcare Group (NHG) have been using a NHG Clinical Practice Guidelines (CPGs) in the form of a flipchart to guide them on the management of the common diseases encountered in the primary health care setting. The content of this flipchart were adapted mainly from MOH's clinical practice guidelines and other sources of evidence based medicine. Clinical practice guidelines have proven to be useful in the practice of evidence based medicine in primary healthcare.¹

This flipchart was developed to guide the busy primary healthcare doctors to practice evidence-based medicine in a practical way, to ensure their patients have the most appropriate quality of care, based on the latest evidence available.

This was the first survey on the flipchart since it has been published by NHG in 2002. The result of this survey will help the organisation in revising the flipchart to be more user friendly, if necessary.

The objective of this study was to determine the usefulness of these Clinical Practice Guidelines in the primary healthcare doctors' clinical practice and their attitude towards evidence-based medicine.

Materials and Methods

There were 2 separate surveys carried out for this study. The first

survey was to all 130 NHG Polyclinics doctors. The self-administered survey forms were distributed to all the doctors though the doctors-in-charge of the nine NHG polyclinics. Response to the survey was voluntary and anonymous. Each clinic collected all the completed survey forms and sent back to the researchers at the end of the survey week.

Concurrently, a second survey was carried out among the GPs who were under the NHG Partners programme. As the researchers required about 100 respondents from the GPs, a randomized list of GPs was generated for this selection process. This was followed by a telephone-administered interview using the same survey form.

The reason to use a telephone administer interview for the GPs instead of a mailed survey form like to the polyclinic doctors was to ensure the response rate to be high and comparable to the polyclinic doctors. This was because based from previous experience; the response rate for self-administered mailed surveys among the GPs was low and thus increased the risk of response bias in the result.

A trained staff conducted the telephone interviews over a period of 2 weeks. The response from the GPs was also purely voluntary and anonymous. If a GP could not contactable for three times on different occasions, it would be deemed as "non-respondent".

At the end of the study period, all questionnaires were centrally collected and audited for completeness and consistency, so that discrepancies and incomplete responses were corrected immediately.

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Responses elicited were encoded and data entered in a database. Scores were assigned after data entry. Statistical package like SPSS was used for univariate, bivariate and regression analysis.

The surveys did not involve any patient's personal information from the doctors. As such, there was no ethical issue to the patients and the doctors. This study merely to obtain feedback on the usefulness of this clinical practice guideline flipchart and to assess the level of evidence-based medicine practiced by the primary healthcare doctors. Approval from the institution's ethics board had also been obtained.

Results

A total of 114 GPs and 74 polyclinic doctors responded to the survey, making the response rate to be 45.4% and 56.9%, respectively. 35.1% of the GPs and 44.8% of the polyclinic doctors have postgraduate qualifications. The respondents work experience varies widely from those who had graduated with basic medical degree more than 40 years ago to those who had just graduated in the last few years.

95% of the polyclinic doctors found the flipcharts useful, compared to only 54.1% of the GPs ($P < 0.05$) (Table 1). Both groups agreed that the use of evidence-based medicine improved patient care in their clinical practice (median score of 8 from a scale of 1 to 10 for both groups, 10 being total agreement).

Those who have favorable attitude towards clinical practice guidelines (score 6 and above out of 10) used the flipchart more often than those who have less favorable attitude. Work experience has no correlation to the use of this flipchart.

Table 1. Percentage of Doctors Who Found the Flipcharts Useful

	Number of doctors	Number who found the flipcharts useful (%)	P
Polyclinic doctors	75	71 (95%)	$P < 0.05$
General practitioners	114	47 (54%)	
Total	189	118	

Discussion

The response rate of about 50% in both surveys was acceptable and comparable to similar surveys conducted previously among primary healthcare doctors in Singapore. The issue of selection biased was minimized with this high response rate.

Although both groups of doctors agreed that the use of evidence-based medicine were important in their clinical practice, GPs in the private practice found the NHG flipchart less useful than their counterparts working in the polyclinics. This could be due to the polyclinic doctors were subjected to regular clinical audits and the flipchart was a useful tool to help them manage patients according to the audit criteria. In a study conducted in UK, it was also found that doctors who were subjected to clinical audits would use clinical practice guidelines more often than those who were not.²

Those doctors who have favorable attitude towards the use of clinical practice guidelines were more willing to use the flipchart than those who do not. Hence, it is important to educate the doctors on the importance and usefulness of clinical practice guidelines to improve the use of this flipchart, which would translate into better care for the patients. Alternate forms of dissemination of these evidence based medicine information like through e-mail could also be considered in the future.³

Acknowledgements

The authors would like to thank A/Prof Shanta Emmanuel, CEO, NHG Polyclinics for allowing them to conduct this survey among the polyclinic doctors and also to NHG Partners for providing a list of GPs for the researchers to interview.

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Best Oral Presentation Award Finalist – General Practice

Attitudes and Perceptions of Physicians to Genetic Testing and Establishment of Genetic Database

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Introduction

The goals of the Human Genome Project are to identify all the genes in human genome and determine the sequence of the 3 billion chemical base pairs that make up the human DNA. Researchers worldwide are now using this data to devise creative applications in an expanding array of fields. However, it has also brought much disquiet and controversies; individuals and society are now beginning

to confront complex ethical and policy issues which, include concepts of anonymity, banking of DNA samples, and the commercialization of genes.¹

The medical profession in most societies is entrusted with the task of looking after the health of its people and safeguarding the interests of patients and the successful implementation of a health initiative (both clinical and research) depends on the support of the medical

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profession. The objectives of this study were to determine the attitudes and perceptions among physicians in Singapore towards genetic testing and the establishment of genetic databanks.

Materials and Methods

Four thousand medical practitioners who were on the mailing list of the Singapore Medical Council were mailed a questionnaire and a covering letter explaining the purpose of the study. They were asked to fill the self-reporting questionnaire adapted from one developed by Hietala et al² and to mail it back to the research institute. This questionnaire consists of 18 self-rating Likert type statements on the following topics (A) attitudes towards gene testing, (B) attitudes towards genetic databanks, and (C) reasons for or against genetic testing. Data from the answered questionnaires was collected and analysed using SPSS v.10 software. Frequency distribution was tested using the χ^2 test.

Results

Of the 4000 physicians, 554 (14%) responded. The sociodemographic factors of the respondents are shown in Table 1.

Attitudes Towards Gene Testing

While 84% of the respondents agree that genetic testing should be made available to those who wish to have information about disease gene they carry, only 20% felt that gene testing should be performed on new-born babies. The majority disagreed with the statement that genetic testing should not be performed at all.

Attitudes Towards Genetic Databanks

Majority of the respondents agreed that genetic banks should be established for all genetic diseases and that they would also personally contribute their genetic material for the establishment of a genetic bank if they or their relatives were suffering from the disease. Most were concerned that the investigators or institutes would derive financial benefits from the genetic bank. Over half of the respondents (67%) agree that the data should be completely anonymised, but more (88%) were of the opinion that donors should be given access to the results of the genotyping.

Table 1. Characteristics of the Group Surveyed for Attitudes and Perceptions to Genetic Database and Genetic Testing (n = 554)

		n	%
Sex	Female	169	30.5
	Male	385	69.5
Age (y)	20-30	136	24.5
	31-40	179	32.3
	41-50	98	17.7
	51-60	87	15.7
	61 and above	52	9.4
Ethnicity	Chinese	506	91.3
	Malay	8	1.4
	Indian	30	5.4
	Others	10	1.8
Religion	Buddhism	55	9.9
	Hinduism	17	3.1
	Islam	13	2.3
	Taoism	3	0.5
	Christianity	330	59.6
	Others	59	10.6
	Atheist	77	13.9
Medical discipline	Surgery	65	11.7
	Psychiatry	26	4.7
	General practitioners	336	60.6
	Obstetrics and gynaecology	21	3.8
	Paediatrics	23	4.2
	Others	6	1.1

Reasons For or Against Genetic Testing

While most (89%) of the respondents felt that people have the right to know about the genes that affect their health as well as that of their children, the majority have concerns about the ethical and legal aspects of genetic testing and genetic banks (Table 2). Eighty-three percent felt that knowledge may lead to discrimination against disease gene carriers, 94% felt that patient's insurance would be jeopardized and 88% were concerned that unexpected family relationships may show up. Just over half (56%) felt that society would save on the cost of treatments as a result of genetic testing.

Discussion

The current survey indicates that the physicians in Singapore have a positive attitude towards genetic testing. The autonomy of the person and the right to self-determination is something which the majority of physicians uphold. The vast majority of respondents was also in favour of establishing a genetic bank and indicated that they would personally donate their own samples. This is not surprising in the case of the physicians practicing in Singapore as the various local pathology departments have long stored tissues samples from autopsies and biopsies, and their training and clinical experience are probably important in influencing their positive perception of such facilities. However, most doctors favour anonymization, expressing concerns about the ramification of personal clinical data being made known to other parties which ranged from fears of discrimination from insurance companies and others, and breakup of relationships. While the majority felt that data should be completely anonymized, they also felt that donors should have access to the results of any

Table 2. Reasons For or Against Genetic Testing

	n	%
Society would save on the costs of treatment of diseases form genetic testing		
Agree	308	55.6
Disagree	138	24.9
Do not know	106	19.1
People have the right to know about their genes which can affect their health and lives		
Agree	496	89.5
Disagree	31	5.6
Do not know	24	4.3
There are more significant public health care problems that must be taken care of first		
Agree	382	69.0
Disagree	104	18.8
Do not know	66	11.9
Knowledge of results may lead to discrimination against disease gene carriers		
Agree	461	83.2
Disagree	66	11.9
Do not know	26	4.7
Patients' insurance coverage could be jeopardized		
Agree	520	93.9
Disagree	12	2.2
Do not know	22	4.0
The genetic material may be used for other tests without the knowledge of the person concerned		
Agree	406	73.3
Disagree	115	20.8
Do not know	32	5.8
The results may end up in the hands of 'outsiders'		
Agree	448	80.9
Disagree	49	8.8
Do not know	55	9.9

subsequent genotyping. The latter would be impossible if there is complete anonymization where the DNA is stripped of its identifiers so that it could not be traced back to the individual person. This apparent contradictory attitude may be due to the inadequacies of the questionnaire which did not explore the degrees of protection that are available to protect the privacy of the donor (for example, the protection of the individual identifying data by independent third parties). This study demonstrates that the physicians in Singapore on the whole favour genetic testing but are concerned about the ethics of such research activities. The continued education and dissemination of information for physicians is important especially with the rapid advance in scientific knowledge which at times outpaces the existing societal ethical and legal norms. It has been suggested that the education of health professionals should also focus on societal, ethical, religious and political issues.³ A mechanism must in put in

place to ensure this as well as to provide a just system for genetic research where the interests of the community is protected without stifling vital research and the development of the biotechnology industry.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Clinical Based)

Psychometric Properties of a New Systemic Lupus Erythematosus-Specific Quality-of-Life Instrument (SLEQOL)

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Introduction

Systemic lupus erythematosus (SLE), a chronic autoimmune illness that usually begins in early adulthood, affects multiple organ systems and may be associated with considerable morbidity and mortality. The onset of complications and response to treatment are generally unpredictable. Thus, SLE impacts the quality of life (QOL) in unique ways and merely measuring the objective outcomes of morbidity and mortality is insufficient.¹ General health instruments have been used to assess QOL in SLE, but disease-specific scales may show greater responsiveness and content validity. We have developed such an instrument (SLEQOL) and we now present its psychometric properties.

Materials and Methods

We developed SLEQOL from scratch. Briefly, a team of Rheumatologists and Nurse Clinicians generated the initial list of 51 QOL items in English. The draft questionnaire was administered to 100 patients. Their responses were subjected to Rasch model and factor analysis and expert review to reduce the item number. SLEQOL was assembled from the reduced list of 40 items, which were divided into 6 subsections.

SLEQOL was administered to a cohort of SLE patients. Data was captured systematically during every study visit. In addition, the SLE Disease Activity Index (SLEDAI), Systemic Lupus Activity Measure (SLAM), Rheumatology Attitudes Index (RAI), MOS 36-Item Short-Form Health Survey (SF-36) were recorded. Patients with recent disease onset within three years were interviewed every three months.

The factors were extracted by principal component analysis and subjected to varimax rotation. We analysed the data with the SPSS Professional Statistics 8.0. We performed Rasch model analysis with BIGSTEPS, a program available at www.winsteps.com. Test-retest reliability was evaluated with the intra-class correlation coefficient (ICC). There is no single best responsiveness statistic for assessing HRQL instruments, so we used four responsiveness statistics to assess the evaluative property of SLEQOL and SF-36 with regards to SLE: Liang's relative efficacy (RE),² Liang's standardized response mean (SRM),³ Kazis' effect size (ES)⁴ and Guyatt's coefficient (GC).⁵

Results

In this cohort of 275 patients, there were 249 females and 26 males, with a mean age was 40.1 ± 13.4 years. The mean age of diagnosis was 31.6 ± 15.2 years. Prospective data at 3-month intervals were obtained from 95 patients and test-retest data from 35.

Face and Content Validity

By inviting 16 Rheumatologists and Rheumatology Nurses and 100 patients to contribute to the initial 51 items, we believe that there had been reasonable face and content validities. The correlation matrix of the SLEQOL against the SF-36, RAI and its helplessness subscale, and the lupus activity scores (Table 1) demonstrates that was minimal association between the SLEQOL and SF-36, SLEDAI or SLAM.

Internal Consistency

Overall, SLEQOL has Cronbach's alpha of 0.95. Cronbach's

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Table 1. The Cross-sectional Correlation Between the SLEQOL (and its Subsections) and SF-36, Rheumatology Attitudes Index (and its Helplessness Index) and the 2 SLE Activity Indices SLEDAI and SLAM

		SLEQOL						
		Summary score	Subsection 1	Subsection 2	Subsection 3	Subsection 4	Subsection 5	Subsection 6
SF-36	Physical functioning	0.112	0.234	0.069	0.066	0.066	0.055	0.042
	Role physical	0.140	0.139	0.106	0.129	0.087	0.067	0.044
	Bodily pain	0.171	0.171	0.098	0.159	0.084	0.090	0.084
	General health	0.127	0.090	0.058	0.112	0.063	0.102	0.080
	Vitality	0.140	0.059	0.087	0.134	0.043	0.127	0.082
	Social functioning	0.107	0.069	0.087	0.059	0.042	0.108	0.055
	Role emotional	0.061	0.056	0.029	0.045	0.030	0.076	0.030
	Mental health	0.125	0.034	0.071	0.096	0.045	0.171	0.082
	Rheumatology Attitudes Index	0.081	0.042	0.016	0.068	0.041	0.053	0.110
Helplessness subscale		0.115	0.053	0.048	0.078	0.046	0.099	0.119
SLEDAI		0.022	0.003	0.018	0.025	0.013	0.010	0.011
SLAM		0.018	0.016	0.010	0.010	0.029	0.007	0.009

Table 2. Rasch Analysis of the 40-item SLEQOL Based on Data Provided by 275 SLE Patients. The Items are Ranked by the Degree of Difficulty, with the Easiest at the Top of the Table

No.	Item	Item Measure	INFIT mean squares	INFIT zstd	OUTFIT mean squares	OUTFIT zstd
2	Shopping	1.01	1.66	2.4	0.74	-0.9
4	Marketing	0.92	1.4	1.6	0.76	-0.9
39	Fears bad news from doctor	0.71	1.59	2.7	1.61	2
40	Consuming more alcohol or tobacco	0.51	1.04	0.3	0.78	-1
3	Turning taps on and off	0.46	1.17	1.1	1.31	1.3
1	Walking outdoors on level ground	0.41	1.06	0.4	0.86	-0.7
32	Wishing others did not know that I have SLE	0.36	1.18	1.2	0.87	-0.6
20	Itchy skin	0.3	1.24	1.6	1.12	0.6
11	Sports	0.25	1.01	0	0.79	-1.1
34	Low self-esteem	0.24	1.04	0.3	0.88	-0.6
24	Fear of needles	0.15	0.89	-1	0.96	-0.2
16	Poor memory	0.14	0.74	-2.4	0.74	-1.5
21	Sore mouth	0.12	1.1	0.9	0.88	-0.7
25	Dietary restrictions	0.12	1.11	1	1.14	0.7
23	Joint pain and swelling	0.08	1.64	4.8	2.4	5.7
33	Friends and colleagues made fun of me	0.05	0.91	-0.9	0.73	-1.7
12	Sex	0	0.86	-1.4	0.72	-1.8
31	Anxiety	-0.01	1.39	3.3	1.77	3.7
9	Missed work or school	-0.03	1.11	1	0.84	-1
27	Inconvenience of frequent clinic visits	-0.04	0.74	-2.8	0.63	-2.6
8	Career or education interference	-0.06	1.09	0.9	0.91	-0.5
7	Work and school performance affected	-0.08	0.9	-1	0.79	-1.4
6	Three km	-0.1	1	0	0.96	-0.2
19	Concentration	-0.1	1.14	1.4	1.4	2.2
26	Inconvenience of daily medications	-0.11	1.05	0.5	1.24	1.4
10	Difficult relationship with friends and relations	-0.11	0.98	-0.2	1.72	3.7
30	Depression	-0.13	0.67	-3.9	0.63	-2.7
29	Feeling low	-0.18	0.67	-4	0.71	-2.1
5	Bathing and drying	-0.18	1.29	2.8	2.23	6
28	Self-consciousness	-0.23	0.66	-4.3	0.63	-2.9
36	Concern about being financial burden to the family	-0.28	1.22	2.3	1.09	0.6
18	Fatigue	-0.31	0.58	-5.7	0.6	-3.4
15	Loss of income	-0.31	0.78	-2.7	0.83	-1.3
22	Sore, painful or stinging skin	-0.32	1.03	0.4	0.93	-0.5
14	Sun	-0.38	1.14	1.5	1.19	1.3
17	Loss appetite	-0.46	0.66	-4.6	0.85	-1.2
13	Social activities	-0.57	1.42	4.5	1.81	5.3
38	Side effects of medicines	-0.58	1.39	4.2	1.33	2.4
37	Medicines don't work	-0.6	1.24	2.7	1.28	2.1
35	Embarrassment	-0.65	1.2	2.2	1.1	0.8

alpha was 0.85 for subsection 1, 0.90 for subsection 2, 0.89 for subsection 3, 0.76 for subsection 4, 0.93 for subsection 5 and 0.86 for subsection 6.

The items in SLEQOL resolve into 8 domains on factor analysis: social and occupational activities, mood and self-image, physical functioning, physical symptoms, self-esteem and the unpredictability of the illness and its response to treatment.

Floor and Ceiling Effect

The ceiling or floor effect occurs when patients perceive that their condition has improved or deteriorated, respectively, beyond what a

QOL questionnaire can measure. The floor effect was more substantial than the ceiling effect in SLEQOL, while the reverse was true for SF-36 (data not shown).

Rasch Analysis of SLEQOL

The Rasch model analysis of SLEQOL is shown in Table 2. The most difficult items for the patients, in decreasing order, were embarrassment, worry that medications do not work, worry about side-effects of medicines and difficulty with social activities. The easiest ones were, in decreasing order, shopping, marketing and fear of receiving bad news from doctors.

Table 3. Responsiveness of the SLEQOL and SF-36. The 4 Responsiveness Statistics Showed Similar Results

		SLEQOL							SF-36							
Responsiveness statistic		Summary score	Subsection 1	Subsection 2	Subsection 3	Subsection 4	Subsection 5	Subsection 6	Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
Liang's standardised response mean	Overall QOL improved	0.44	0.31	0.51	0.59	0.23	0.37	0.23	0.00	0.11	0.13	0.31	0.11	0.21	0.13	0.12
	Overall QOL unchanged	0.21	0.13	0.27	0.00	0.05	0.07	0.16	0.29	-0.08	-0.08	-0.10	0.01	0.02	0.02	-0.01
Kazis' effect size	Overall QOL improved	0.33	0.29	0.39	0.48	0.25	0.33	0.19	0.00	0.11	0.12	0.26	0.11	0.20	0.14	0.12
	Overall QOL unchanged	0.15	0.12	0.19	0.00	0.04	0.07	0.17	0.20	-0.08	-0.07	-0.09	0.01	0.01	0.02	-0.01
Guyatt's coefficient	Overall QOL improved	0.37	0.31	0.43	0.57	0.22	0.35	0.22	0.00	0.12	0.12	0.26	0.11	0.18	0.15	0.12
	Overall QOL unchanged	0.15	0.12	0.19	0.00	0.04	0.07	0.17	0.20	-0.08	-0.07	-0.09	0.01	0.01	0.02	-0.01
Liang's relative efficacy (excluding the SLEQOL subsections)		1.00	-	-	-	-	-	-	0.04	0.03	0.12	0.32	0.03	0.06	0.06	0.01
Liang's relative efficacy		0.97	0.17	0.73	1.00	0.20	0.54	0.24	0.03	0.03	0.11	0.31	0.03	0.06	0.06	0.01

Test-retest Reliability

Thirty-five patients with stable disease were interviewed twice in two weeks to determine the test-retest reliability. The ICC was 0.86 for the summary score. For subsections 1 to 6, the ICCs were 0.72, 0.56, 0.74, 0.66, 0.68 and 0.82 respectively.

Responsiveness

All patients who completed the SLEQOL in the subsequent visits beyond the baseline were asked to rate their global change in QOL using a scale of integers from -7 to +7. In all, 119 data pairs from 95 patients were available for analysis. All the 4 responsiveness statistics show that the SLEQOL is more sensitive than any of the 8 domains of the SF-36 (Table 3). Subsections 3, 4 and 5 of the SLEQOL were the best indicators of change of QOL, superior to the summary score and all the domains of the SF-36. They were very sensitive to change in the QOL and they did not vary when the patients rated their QOL as unaltered.

Conclusion

We have developed a new 40-item SLEQOL in English and showed that it is valid for use in SLE patients in Singapore. It is

internally consistent and has reasonable test-retest reliability and floor and ceiling effect. It offered better content validity and responsiveness to change than the SF-36.

Acknowledgements

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Perinatal Depressive Disorders in Singaporean Women and Their Partners

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Introduction

Depression is a leading cause of disease burden in women aged 15 to 44 years in both developed and developing countries,¹ affecting up to 13% of women² and often having onset during the reproductive years. Apart from the adverse consequences for the women involved, maternal depression has been shown to have profound effects on the relationship between mother and child and on the child's emotional, behavioral and cognitive development.¹

The prevalence of postnatal depression in Singapore is unknown,³ although studies in Asian populations have suggested that it is no longer as uncommon as previously reported.⁴ Our aim was to study the prevalence of depressive disorders in women and their partners in Singapore, as well various psychosocial variables associated with perinatal depression.

Materials and Methods

Five hundred fifty-nine women and 308 men were recruited antenatally from obstetric clinics at National University Hospital between July 2002 and July 2004. Following baseline evaluation, subjects were followed up at 6 weeks postnatally.

Evaluation for depressive disorders used a two-stage design, with women and men scoring above cut-offs on the Edinburgh Postnatal Depression Scale (EPDS)⁵ going on to a Structured Clinical Interview for DSM-IV (SCID-IV). The 10-item, self-report EPDS has been well-validated in several languages for use as a screening tool for both antenatal and postnatal depression and is easy to administer. The mood disorders that were specifically looked for in this sample were that of major depressive disorder (MDD), minor depressive disorder and dysthymia.

We also examined risk factors that were commonly reported in previous studies of postnatal depression. Various domains of risk factors included:

- demographic and socio-economic factors such as age, marital status, number, age and gender of other children, educational level, employment status, and household income, whether living with extended family or not
- medical and psychiatric history: past depressive episodes, previous miscarriage, previous induced abortion, obstetric complications
- interpersonal relationships: marital and sexual satisfaction, ratings of emotional and practical support (also known as instrumental support) by various family members such as one's partner, parents, parents-in-law as well as by domestic helpers
- sociocultural factors such as perceived potential conflicts with other family members, confinement practices, as well as miscellaneous associations such as breastfeeding at 6-weeks postnatally.

Statistical analysis was done using SPSS version 12.0 for Windows OS). Univariate analysis for independent variables was performed using independent Student's *t*-test for continuous variables and chi-square tests for dichotomous and categorical variables. Risk ratios were calculated with 95% confidence intervals.

Logistic regression analysis was used to estimate the univariate odds ratios and their corresponding 95% confidence intervals. Multiple logistic regression was used to explore the important independent associations between variables and mood disorders after adjustment for confounders.

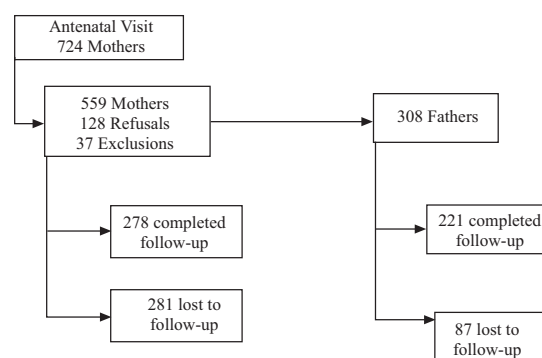


Fig. 1. Summary flowchart of patients who were recruited antenatally, refusals, exclusions and patients lost to follow-up at 6 weeks.

Results

Limited information about the refusals was obtained from their patient registration data; however, comparison with baseline data from the 559 patients who agreed to participate in the study showed that the 2 groups appeared to be comparable in terms of age and ethnic make-up.

Statistical analysis done on the mothers who dropped out at the 6-week postnatal interview showed that the 2 groups did not differ significantly from each other in all the studied variables except for ethnic group.

12.2% of women and 4.55% of men were diagnosed as having a depressive illness antenatally. This fell to 6.8% of women and 1.81%

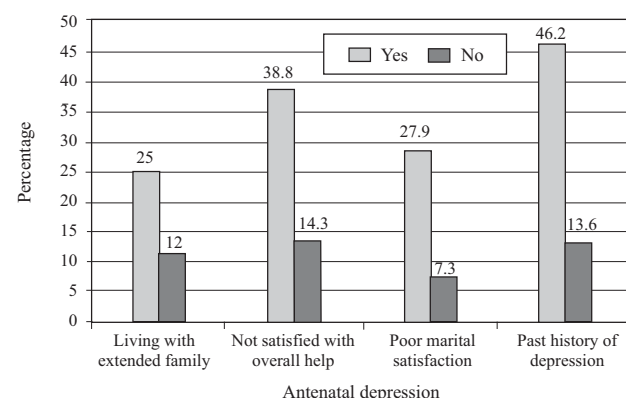


Fig. 2. Significant predictors of depressive disorders in antenatal women using logistic regression modelling.

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of men at 6 weeks postnatally. When considering major depressive disorder alone, the percentage of mothers suffering from MDD stayed fairly stable at 4.29% and 4.32% respectively. The percentage of fathers suffering from MDD was too small for meaningful analysis. Owing to the high drop-out rates, one must be careful not to over-interpret the results. However, given that the baseline characteristics of follow-ups and drop-outs did not differ, it is striking to note the drop in minor depressive disorder rates but not major depressive disorder rates across delivery.

Significant factors associated with antenatal depression in women included marital dissatisfaction, dissatisfaction with overall help given, past history of depression and living with one's own parents.

Factors assessed antenatally which were not statistically significant were: the gender of the baby, young age (less than 25 years old), ethnic group, whether the patient was undergoing confinement or not, past history of abortion or miscarriage, employment status, educational level, household income and practical help scores.

At 6 weeks postnatally, multiple logistic regression analysis showed that the women who suffered from depressive disorders were more likely to have poor overall help, to have poor marital satisfaction, to have brought their child to the doctor for 3 or more non-routine visits and to have lower practical help scores than those who were not depressed.

Breastfeeding status (either full or supplemented) at 6 weeks postnatally was not significantly associated with depressive disorder in our sample.

Factors associated with depressive disorders in partners included whether they thought their wives were likely to have significant conflict with relatives over childcare. Other factors did not reach

statistical significance owing to the suboptimal sample size of partners with depressive disorders.

Conclusions

Perinatal depressive disorders are not uncommon in Singaporean women. Though there is a drop in their prevalence at 6 weeks postnatally, a significant minority of women continues to suffer from depressive disorders, and prevalence rates of major depressive disorder remain constant both antenatally and postnatally. Psychosocial factors particularly associated with depressive disorder included marital dissatisfaction and dissatisfaction with overall help.

Screening and follow-up for depression in at-risk women are recommended to ensure early detection and treatment of this debilitating condition. Given the loss to follow-up in postnatal populations, screening could be targeted at antenatal women and in women who bring their infants for 3 or more non-routine clinic visits during the postpartum period.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Clinical Based)

Improved Outcome with Intensive Chemotherapy in Paediatric Acute Myeloid Leukaemia

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Introduction

Acute myeloid leukaemia (AML) accounts for 20% of childhood acute leukaemia.¹ As recently as the 1990s, 2-year disease-free survival from AML in Singapore was only 30%.²

Since September 1996, the United Kingdom Medical Research Council's 10th AML trial (UKAML-10) protocol has been adopted in Singapore. UKAML-10 is a highly intensive, near-myeloablative regimen designed to improve the previously dismal results of AML therapy for both children and adults. Although superiority of the UKAML-10 protocol in developed countries can be demonstrated by comparing published data,³⁻⁴ its value in countries with more limited resources and its toxicity profile have yet to be documented.

We review our institutional experience with UKAML-10 and

previous regimens in a consecutive series of children with AML. Comparing their survival and toxicity profiles will ascertain if survival benefits accrued after switching protocol outweigh the risks.

Materials and Methods

Patients

Characteristics of the 34 patients treated between April 1988 and December 2003 are summarised in Table 1.

Diagnosis of Leukaemia

This was established with standard methodology.

Treatment

Prior to September 1996, 10 children received the POG-8498 protocol and nine received other regimens.

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Table 1. Patient Characteristics

Parameter	No. of children	% of children
Gender		
Male	21	62
Female	13	38
Age (years)		
<2	12	35
2-7	14	41
>7	8	24
Type of AML		
De novo	31	91
Secondary	0	0
MDS-AML	3	9
FAB subtype		
M0/M1	0	0
M2	7	21
M3	2	6
M4	7	21
M5	4	12
M6	0	0
M7	13	38
unclassifiable	1	3
Extramedullary disease		
None	30	88
CNS	0	0
Chloroma	4	12
White cell count ($\times 10^9/L$)		
<10	15	44
10-29	10	29
30-99	6	18
>100	3	9
Cytogenetic group		
Favourable – t(8;21), t(15;17), inv(16)	8	24
Intermediate – all other cytogenetics	14	53
Adverse – monosomy 5/7, del(5q), 3q abnormalities, complex karyotype	4	12
Unknown	4	12
Down syndrome		
Yes	7	21
No	27	79
Chemotherapy protocol		
UKAML-10	14	41
Others	20	59

AML: acute myeloid leukaemia; CNS: central nervous system; UKAML: United Kingdom Medical Research Council 10th AML Trial

Of 15 children treated from September 1996 onwards, 14 received UKAML-10 (four courses in all: two remission induction courses with ADE [cytarabine, daunorubicin, etoposide] given for 10, 3 and 5 days and 8, 3 and 5 days for course 1 and 2 respectively; 2 consolidation courses consisting of MACE [amsacrine, cytarabine, etoposide] and MidAC [mitoxantrone, cytarabine]). One received another protocol.

Definitions

Complete remission (CR) was defined by a normocellular bone marrow aspirate containing <5% blasts and normal maturation of other marrow elements.

Overall survival (OS) is time from diagnosis to death; event-free survival (EFS) is time from diagnosis to first event (relapse, death in CR or death without CR); disease-free survival (DFS) is time from CR to any event (relapse or death in CR).

Statistical methods

Remission rates were compared using chi-squared tests. Survival data were computed using Kaplan-Meier analysis. Prognosticators analysed included: presenting white blood cell count (WBC); cytogenetics; French-American British (FAB) subtype; achievement of CR after course 1; age; and glutathione s-transferase (GST) T1/M1 genotype. The Cox proportional hazard model was used to adjust for influence of protocol on survival. Surviving patients were censored

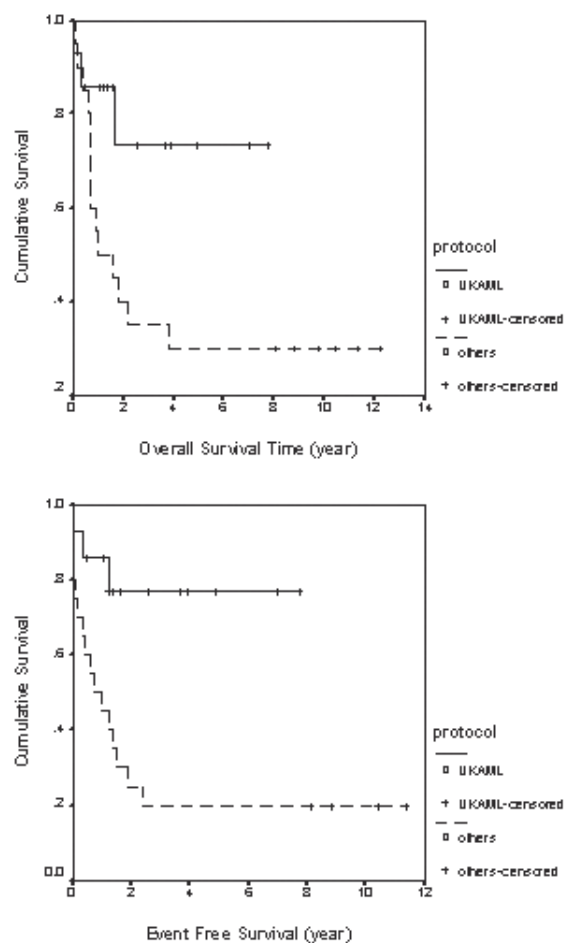


Fig. 1. Overall survival and event-free survival for UKAML-10 vs other protocols.

at 31 May 2004, when follow-up was up-to-date for all children.

Results

Outcomes

At time of analysis, 17 were dead from disease, and 17 patients alive among whom 2 relapsed. Median follow-up was 1.6 years (range, 0-12.3).

UKAML-10-treated patients had significantly better 2-year OS, EFS and DFS (73.5% vs 40.0%, 77.1% vs 25.0%, 83.1% vs 38.5%; $P = 0.039$, $P = 0.005$, $P = 0.023$ respectively) [Fig. 1] and were likelier to achieve CR than non-UKAML patients (92.9% vs 65.0%, $P = 0.102$, odds ratio = 7.0). Among patients achieving CR, UKAML-10-treated patients were significantly likelier to achieve CR after only 1 cycle of chemotherapy (84.6% vs 38.5%, $P = 0.016$, odds ratio=8.8).

Toxicity of Chemotherapy

Haematologic toxicity: There was no significant difference between UKAML-10 and other protocols in median duration of hospitalisation for neutropaenic fever, duration of neutropaenia or thrombocytopaenia ($P = 0.920$, $P = 0.724$, $P = 0.479$ respectively; Mann-Whitney U test).

Non-haematologic toxicities: These were graded by current NCI criteria. UKAML-10 and other protocols did not differ much in gastrointestinal toxicity, cardiotoxicity, hepatotoxicity, and nephrotoxicity. Four non-UKAML patients died from sepsis, versus none from the UKAML-10 group.

Prognostic Factors

Presenting WBC: WBC > 30 × 10⁹/L predicted a lower OS ($P = 0.009$) and EFS ($P = 0.01$), independent of protocol.

Cytogenetics: Adverse cytogenetics predicted for poorer OS ($P = 0.023$) and EFS ($P = 0.014$) compared to intermediate risk cytogenetics, adjusting for protocol.

Other prognosticators: FAB subtype, achievement of CR after course 1, and age at presentation did not show any significance in 2-year OS, EFS or DFS. GST genotypes are discussed below.

Cost

Median cost of chemotherapy was S\$4,026 (range, 2013-8168) for UKAML-10 patients and S\$1624 (range, 809-6929) for non-UKAML patients. The disparity ($P = 0.003$) was due to higher doses of existing drugs, such as daunorubicin and etoposide, as well as the introduction of newer, more expensive drugs such as amsacrine and mitoxantrone, in UKAML-10.

Discussion

The intensity of UKAML-10 chemotherapy is greater than in other protocols, in terms of the duration and dosage of individual courses and total number of courses given. This appears to translate into a significant survival advantage. Given the lower relapse rate and proportion of deaths in relapse for UKAML-10, it is likelier that improved survival is primarily due to the protocol itself rather than advances in supportive care.

Interestingly, the more intensive UKAML-10 chemotherapy did not result in greater haematologic toxicity. Although this was probably confounded by increased G-CSF use, the latter is unlikely to affect overall outcome as meta-analysis shows that G-CSF only shortens duration of neutropaenia without reducing overall mortality.⁵

Regarding cardiotoxicity, resting fractional shortening (FS) on echocardiography is known to be inversely related to cumulative anthracycline dose (rate of decline 1.2%/100mg/m²).⁶ Three patients (9% of total) experienced a 10% drop in FS after a 250mg/m² anthracycline dose (corresponding to a 4%/100mg/m² rate of decline), possibly reflecting greater susceptibility to anthracycline cardiotoxicity in the Singapore population.

In the original UKAML-10 trial, lower presenting WBC, favourable

cytogenetics, M5 FAB subtype and younger age predicted for better OS and EFS. Only the first 2 were significantly associated with survival in our study.

Patients with GST wild-type and less intensive chemotherapy (ie, non-UKAML protocols) showed a trend towards lower survival, compared to GST-null patients. This may have implications for treatment stratification; GST-positive children (efficient metabolisers of chemotherapeutics) will require more intensive induction therapy to ensure a durable remission.

Although overall cost of managing each patient was not analysed, UKAML-10 is probably much cheaper despite the high cost upfront, as we no longer resort to more expensive modalities like bone marrow transplantation to salvage relapse patients.

Conclusion

The quantum leap in survival from childhood AML over the past decade validates UKAML-10 as an effective chemotherapy regimen. Future studies should dissect individual components of UKAML-10 to elucidate their relative efficacy and fine-tune therapy. For a disease once considered uniformly fatal, the synergy of treatment optimisation and translational research heralds a much improved prognosis.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Laboratory Based)

Effects of Inhibitors of the Tyrosine Signalling Cascade on Antigen Challenge of Guinea Pig Airways *in vitro*

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Introduction

Mast cell has been implicated to play a pivotal role in asthma because mast cell degranulation induced by the cross-linking of the high-affinity IgE receptors (FcεRI) releases a panel of inflammatory mediators such as histamine, leukotrienes, and cytokines which can initiate, coordinate, and perpetuate the allergic inflammatory responses. Cumulative evidence obtained from rat basophilic leukaemia

cell line (RBL-2H3) and bone marrow-derived mast cells shows that activation of src-related kinase Lyn and 72-kDa Syk tyrosine kinase is the earliest detectable signalling response to FcεRI cross-linking. This is followed by the activation of downstream signalling molecules including phospholipase Cγ, protein kinase C, phosphatidylinositol-3-kinase (PI3K), and mitogen-activated protein kinase (MAPK), which eventually leads to mast cell degranulation (Fig. 1).

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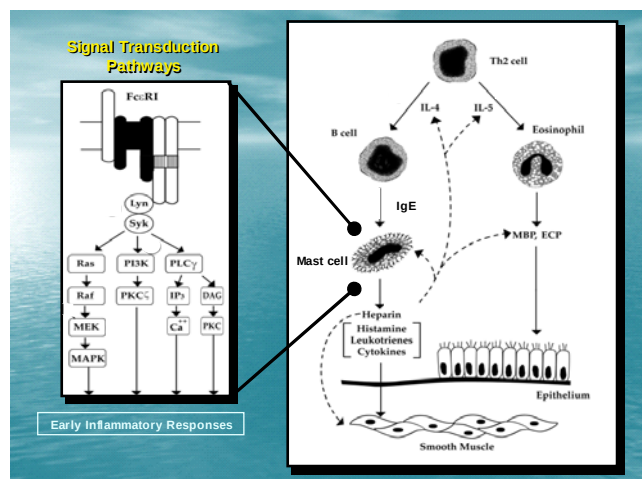


Fig. 1. Schematic diagram showing the immunopathogenesis of asthma.

In mammalian systems, three major groups of MAPK have been identified: p42/44 MAPK (also referred to as extracellular signal-regulated kinase 2 (ERK2) and ERK1, respectively), p38 MAPK and c-Jun NH₂-terminal kinase (JNK). It has been shown that IgE-mediated FcεRI cross-linking in various mast cell cultures caused activation of all three MAPKs. However, as the time-course of JNK activation did not precede that of mediator release, it was believed that only p42/44 MAPK and p38 MAPK were potentially involved in IgE-induced mast cell degranulation. Because mast cell degranulation is the hallmark of immediate-type hypersensitivity reaction, which is also the major mechanism for a variety of allergic diseases such as bronchial asthma, it is imperative to examine the effects of protein tyrosine kinase inhibitors on an *in vitro* model of allergic asthma.

The present study compared the effects of 4 tyrosine kinase inhibitors: Piceatannol, a Syk-selective tyrosine kinase inhibitor; U0126 [1,4-diamino-2,3-dicyano-1,4-bis(aminophenylthio) butadiene], a selective p42/p44 MAPK kinase inhibitor and p38 MAPK inhibitors PD169316 [4-(4-fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-1H-imidazole] and SB220025 [5-(2-amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole] in an *in vitro* model of allergic asthma.

Materials and Methods

Guinea pigs were sensitised by intra-peritoneal injections of ovalbumin (OVA). They were subsequently sacrificed and bronchial rings were contracted to 60 mM KCl, and this contraction was defined as the maximum tissue response to which all subsequent anaphylactic contractions were compared. To evaluate the role of tyrosine kinase in mediating anaphylactic bronchial smooth muscle contraction, the inhibitors were each pre-incubated with bronchial rings 30 min before exposure to ovalbumin. To examine potential direct receptor antagonistic effects of the inhibitors, we also studied bronchial contraction directly induced by histamine, LTD₄, or KCl in the presence and absence of the inhibitors. To study the potential direct smooth muscle relaxant effects, the inhibitors were individually added at peak ovalbumin-induced anaphylactic bronchial contractions, with 1 μM salbutamol used as a positive control.

To determine the inhibitory effects on antigen-induced release of histamine and peptidoleukotrienes, each of these inhibitors was pre-incubated for 30 min before ovalbumin challenge. To determine if these inhibitors have any direct inhibitory effects on the *de novo* synthesis of peptidoleukotrienes such as inhibition of 5-lipoxygenase

activity, 70 μM exogenous arachidonic acid was added to lung fragments alone or with ovalbumin challenge for 10 min in the presence and absence of these inhibitors. Diffusates were then collected and stored at -70°C until assay. Histamine and peptidoleukotrienes release from lung samples in response to ovalbumin was determined using an enzyme-linked immuno-sorbent assay (ELISA). Optical density was determined using a microplate reader (Tecan, Austria) at 450 nm. Samples were assayed in duplicate.

Results

Pre-treatment with piceatannol and U0126 produced minor reduction in peak ovalbumin-induced bronchial contraction but markedly facilitated relaxation of the anaphylactically contracted bronchi. SB 220025 and PD 169316 did not suppress ovalbumin-induced peak bronchial contraction or facilitate its relaxation.

Piceatannol and U0126 did not inhibit bronchial contractions induced by KCl, histamine or leukotriene D₄-induced bronchial contraction. Correspondingly, U0126 produced slight reduction in ovalbumin-induced release of histamine but significant inhibition on the release of peptidoleukotrienes from lung fragments. Piceatannol, at 30 μM and above, significantly ($P < 0.05$) prevented ovalbumin-induced release of both histamine and peptidoleukotrienes from lung fragments.

Exogenous arachidonic acid-induced release of peptidoleukotrienes was not blocked by U0126. Piceatannol did not inhibit exogenous arachidonic acid-induced release of peptidoleukotrienes from lung fragments.

SB220025 and PD169316 failed to show any inhibitory effect on ovalbumin-induced release of histamine and peptidoleukotrienes. Our findings indicate that inhibition of Syk tyrosine kinase and p42/44 MAPK kinase, but not p38 MAPK, significantly reduced allergen-induced release of mediators leading to rapid relaxation of anaphylactic bronchial contraction.

Discussion

Inhibition of Syk by piceatannol, a widely reported Syk-selective inhibitor, has been shown to inhibit FcεRI-mediated histamine and serotonin release from isolated mast cells and basophils. Piceatannol has been shown to potently inhibit the activity of Syk at concentrations that have little or no effect on Lyn, Fyn and cyclooxygenase. Our findings show that inhibition of Syk by piceatannol can block mast cell degranulation in airways *in vitro*, consistent with the findings observed in isolated mast cells, and a substantial reduction in histamine release is required in order to significantly attenuate the peak anaphylactic bronchial contraction. Results of the mediators release study showed that piceatannol did not block exogenous arachidonic acid-induced release of peptidoleukotrienes from lung fragments, suggesting that the inhibitor does not have direct effect on 5-lipoxygenase activity. Therefore the substantial reduction in peptidoleukotrienes release by piceatannol via Syk inhibition is likely linked to the rapid relaxation of the ovalbumin-induced anaphylactic bronchial contraction.

Our work with U0126 shows that it did not substantially inhibit the amplitude of ovalbumin-induced bronchial contraction. This observation corroborates well with a slight 20% inhibition of ovalbumin-induced histamine release from lung fragments by U0126. This suggests that p42/44 MAPK plays only a minor role in IgE-mediated histamine release from mast cells. In contrast, U0126 significantly facilitated relaxation of the anaphylactically contracted bronchi in a concentration-dependent manner. Since U0126 did not affect direct histamine-, LTD₄- or KCl-induced bronchial contraction, the rapid relaxant effect is not mediated by non-specific receptor antagonism or voltage-dependent calcium channel blockade. Instead,

U0126-mediated bronchial relaxation is likely associated with the substantial suppression of ovalbumin-induced release of peptidoleukotrienes as shown in the mediator release studies in lung fragments.

Inhibitors of the p38 MAPK such as SB239063 and SB220025 have been shown to reduce inflammatory cytokine production and eosinophil infiltration into the lungs in animal models of asthma, and inhibited angiogenesis in murine model of rheumatoid arthritis. Although IgE cross-linking on mast cells has been shown to activate p38 MAPK, inhibition of p38 MAPK by SB203580 failed to block the release of histamine, arachidonic acid and peptidoleukotrienes from cultured mast cells. In line with the cell culture findings, our results show that both SB220025 and PD169316 failed to inhibit ovalbumin-induced bronchial contraction and the release of histamine and peptidoleukotrienes from lung fragments. These findings together with our *in vitro* results clearly confirm that p38 MAPK does not play a role in early asthmatic response upon mast cell degranulation.

The detailed signaling mechanisms mediating FcεRI-induced mast cell degranulation have been unraveled and a signal transduction-based approach offers a specific strategy to block mast cell activation. Our present findings show that Syk and p42/44 MAPK inhibitors,

but not p38 MAPK inhibitors, facilitated relaxation of constricted airways by preventing antigen-induced release of mediators, indicating that they may have therapeutic potential for asthma.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Laboratory Based)

Serum Transferrin Receptor Levels in the Normal Population and Subjects With Iron Deficiency and Thalassaemia Trait

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Introduction

Traditional biochemical markers used in iron deficiency diagnosis have pitfalls, especially in the presence of inflammation. Serum transferrin receptor level (sTfR) is helpful as it is unaffected by inflammation. However, disease states like hemolytic anemias and ineffective erythropoiesis can confound the results. We aimed to study how demographic factors affect sTfR and how thalassaemia trait and iron deficiency interact to affect sTfR.

Materials and Methods

From May 1999 till December 2001, blood sent for thalassaemia screening from patients in Tan Tock Seng Hospital were concurrently checked for sTfR, serum iron, serum transferrin (or total iron binding capacity (TIBC)) and serum ferritin. The subjects' age, sex and the closest full blood count (FBC) were recorded. As control, 140 health screening participants were checked for sTfR, serum ferritin and FBC.

sTfR levels were quantitated using N Latex sTfR assay (Dade Behring, Liederbach, Germany) on a BN100 nephelometer (Dade Behring, Liederbach, Germany). Transferrin was similarly measured by an immunonephelometric assay. Ferritin was measured using a microparticle enzyme immunoassay on the Abbott AxSym system. Iron and TIBC was measured using the FerroZine photometric method on Roche/Hitachi analysers.

A parallel study analysing the accuracy of iron parameters in diagnosing iron deficiency in tertiary hospital patients found that an

optimal ferritin level of <60 µg/L or a transferrin saturation level of <7% has a positive predictive value of 94%. Iron deficiency diagnosis for study subjects is based on similar criteria. Iron deficiency among controls was diagnosed only if ferritin <30 µg/L, based on normal reference ranges.

Full blood count was measured on the Gen.S™ automated analyser (Coulter Corporation, Miami, Florida). Thalassaemia screening included analysis by haemoglobin electrophoresis in alkaline pH on cellulose acetate plates (Helena Laboratories, Beaumont, Texas) and HbA₂ quantitation by high performance liquid chromatography using the Variant™ hemoglobin testing system (Bio-Rad Laboratories, California). HbA₂ levels between 3.8% and 8% with appropriate red cell indices were considered diagnostic of β thalassaemia trait. Samples demonstrating occasional HbH inclusion bodies using Methylene Blue were classified as a thalassaemia trait (mainly 2 gene deletions).

Statistical analyses (correlation coefficient and Student's *t*-test) were done using Microsoft™ Excel. Significance testing of the correlation coefficient was done using an online calculator from Vassar College.

Results

Controls

Among 107 controls (61 males, 46 females) with normal ferritin levels, the mean sTfR level (±2 SD) is 1.20 ± 0.47mg/L. There is no

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difference in mean sTfR levels between males (1.21 mg/L) and females (1.18 mg/L) ($P = 0.41$), even though ferritin levels is significantly higher among males. A weak negative correlation exists between age and sTfR levels ($r = -0.21$, $P = 0.02$), stronger among males ($r = -0.26$, $P = 0.02$) than females ($r = -0.12$, $P = 0.21$).

Thirty-three controls had subclinical iron deficiency (normal hemoglobin and mean corpuscular volume, low ferritin levels). Their mean sTfR level (1.39 mg/L) was significantly higher than that of the above 107 controls ($P = 0.003$). Nonetheless, the large overlap in sTfR levels between these two groups precludes its use in iron deficiency screening.

Subjects

There were 432 subjects with different combinations of α , β -thalassaemia traits and iron deficiency (Table 1). Subjects with isolated iron-deficiency and isolated β -thalassaemia trait have significantly higher sTfR levels than subjects with no pathology ($P < 0.001$). This difference was not found amongst subjects with isolated α -thalassaemia trait.

Table 1. Distribution of Subjects with Iron Deficiency/Thalassaemia Trait

	n	Mean sTfR (mg/L)	Mean ferritin (μ g/L)
No pathology (non iron deficient, non thalassaemia trait)	23	1.54	384
Isolated α -thalassaemia trait	100	1.48	230
Isolated β -thalassaemia trait	112	2.06	377
Isolated iron deficiency	116	4.64	10.5
Ferritin $\geq 10 \mu$ g/L	36	3.82	24.8
Ferritin $\geq 15 \mu$ g/L	26	3.74	30.0
Combined α -thalassaemia trait and iron deficiency	56	2.80	22.6
Combined β -thalassaemia trait and iron deficiency	25	2.57	30.1
Total	432		

Subjects with combined thalassaemia trait and iron deficiency have significantly lower sTfR levels than subjects with isolated iron deficiency ($P < 0.001$). This might be partly due to the significantly greater degree of iron deficiency, as measured by ferritin, amongst isolated iron deficient subjects ($P < 0.001$). Adjustment for comparability was done to the ferritin levels by considering isolated iron deficient subjects with ferritin levels $\geq 10 \mu$ g/L or $\geq 15 \mu$ g/L in the comparison with combined α -thalassaemia/ iron deficiency or β -thalassaemia/ iron deficiency respectively. Re-analysis showed that the differences in the sTfR levels persisted ($P = 0.02$ for both comparisons).

Discussion

In this study, we analysed how physiological factors influence sTfR levels. One published study measured sTfR in 56 normal adults and found a slight but insignificant reduction of sTfR with age.¹ Our study of 107 normal controls showed a weak but significant negative correlation between age and sTfR levels which is not due to variations in ferritin levels. Whether this weak negative correlation between sTfR levels and age contributes to the physiological decline in hemoglobin levels with age requires further investigation.

Among subjects, iron deficiency significantly raises the sTfR levels, as shown in other studies.^{2,3} A Greek study showed that β thalassaemia trait raises sTfR levels in proportion to the degree of ineffective erythropoiesis.⁴ We also showed that β thalassaemia trait

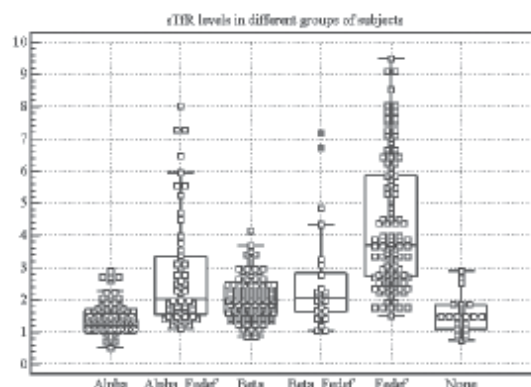


Fig. 1. sTfR levels in different groups of subjects. 3 outliers in Fe def group not included.

raises sTfR levels but correlation of sTfR levels with the degree of ineffective erythropoiesis was not found (results not shown). In our study, α thalassaemia trait does not raise sTfR levels. This is at variance with South Pacific island study where sTfR levels were found to increase in proportion to the number of α globin gene mutations.⁵ Nevertheless, this study was done in children and applicability to an adult inpatient population is uncertain.

We demonstrated that both thalassaemia traits attenuate the erythron's transferrin receptor upregulating response to the absence of iron. The mechanism behind this phenomenon is unknown but it is possible that the statistical adjustment made in our study is not reflected biologically. Whether this will translate into a clinically reduced response to iron therapy in patients with concurrent thalassaemia trait and iron deficiency needs further investigation.

The utility of sTfR as a single marker in the diagnosis of iron deficiency can be predicted in Figure 1. The significant overlap, especially between iron deficient subjects and non-iron deficient subjects with thalassaemia trait will reduce accuracy of this marker.

Conclusion

Age negatively correlates with sTfR among males. β -thalassaemia trait but not α -thalassaemia trait causes a rise in sTfR levels. Thalassaemia traits attenuate the increase in sTfR caused by iron deficiency. This will affect the accuracy of sTfR in iron deficiency diagnosis in this group of patients.

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***Escherichia coli*-asparaginase (Elspar) is Superior to *Erwinia*-asparaginase (Erwinase) in Childhood Acute Lymphoblastic Leukaemia (ALL) Induction – An Early Response Study Using Minimal Residual Disease (MRD) Markers**

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Introduction

L-asparaginase hydrolyses L-asparagine, causing its depletion in the blood. It is one of the most important drugs responsible for the highly successful therapy of childhood acute lymphoblastic leukaemia (ALL). Malignant lymphoblasts, unlike normal cells, are unable to synthesise asparagine, hence its depletion leads to lymphoblast apoptosis.^{1,2}

L-asparaginase in clinical use is derived from either *Escherichia coli* (Elspar) or *Erwinia chrysanthemi* (Erwinase). Its adverse effects, including coagulopathy, hepatotoxicity and pancreatitis,³ result primarily from the effect of asparagine depletion on normal protein synthesis. Different sources of asparaginase differ in pharmacodynamics and pharmacokinetics, with Elspar believed to be more potent and longer-acting, while Erwinase causes fewer allergies and toxicities.^{1,2} Initial widespread switches from Elspar to Erwinase were blamed for poorer results in some groups; however, direct proof of the inferiority of Erwinase has not been demonstrated.

Sensitive molecular techniques allow detection of minimal residual disease (MRD) levels as low as 1 leukaemia cell in a background of 10,000 normal cells, compared to 5 in 100 cells with conventional microscopy, allowing accurate following of the kinetics of early leukaemia cell kill.⁴ We utilised these techniques to compare the efficacy of Elspar versus Erwinase in killing lymphoblasts *in vivo* in childhood ALL patients.

Materials and Methods

Patients and Samples

Using MRD as an indicator of treatment response, we compared the induction regimens of 2 childhood ALL trials: NUH (1992-1996) and MA-SPORE (2002-ongoing). The NUH protocol used either Erwinase or Elspar 10,000 IU/m² twice a week, while the MA-SPORE uses Elspar 7500 IU/m² bi-weekly. Dosages of other drugs were similar.

A total of 110 newly diagnosed precursor-B ALL patients from NUH (n=45) and MA-SPORE (n=65) protocols with data available regarding the L-asparaginase source were included; 21 were on Erwinase, 89 on Elspar. The median age was 5.3 years (range, 0.36 to 13.8), the median presenting total white count (TWC) 11.5x10⁹/L (range, 1.08 to 729). DNA was extracted from bone marrow mononuclear cells (MNC).

Minimal Residual Disease Determination

Antigen receptor gene rearrangements were analysed by PCR as described.⁴ Sequences were compared against the human germline (<http://www.ncbi.nlm.gov/BLAST/>) and patient-specific primers constructed.

Southern Blot (SB): Patient DNA was amplified by PCR, transferred

onto a nylon membrane and hybridised with the specific radio-labelled oligoprobe.

RQ-PCR: The LightCycler (Roche Diagnostics) was used, with patient-specific primers and Taqman probes.

In both methods, the remission sample was compared against a standard dilution of the diagnostic sample, with water and MNC as negative controls.^{3,5}

Patients were stratified into High Risk (HR: $\geq 10^{-2}$); Intermediate Risk (IR: 10^{-2} - 10^{-4}); and Standard Risk (SR: $\leq 10^{-4}$) according to level of MRD at week 5.

Toxicity Data

Data regarding adverse effects were collected from casenotes. Toxicity was graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (<http://ctep.cancer.gov/reporting/ctc.html>).

Results

SB And RQ-PCR Correlation

Blinded comparison between SB and RQ-PCR gave 100% (11/11) correlation between the 2 methods of MRD detection, with both reaching at least 10⁻⁴ sensitivity.

Patient Composition

The composition of the 2 groups were identical; specifically, 71% of both Erwinase and Elspar-treated patients are low risk by NCI criteria, thus there is unlikely to be a selection bias (Table 1).

MRD Risk Stratification

Samples at both diagnosis and end of induction were available for 62% (13/21) of Erwinase-treated patients and 84% (75/89) of Elspar-treated patients, allowing MRD determination for an overall 80%.

Significantly more Erwinase-treated patients were MRD-stratified into high risk compared to Elspar-treated patients (31%, 4/13 vs 8%, 6/75, $P = 0.037$; OR = 5.11, 95% CI, 1.3-20.6). Within the Elspar group, stratification of NUH or MA-SPORE patients was not significantly different (HR: 21%, 3/14 vs 5%, 3/58, $P = 0.13$), showing that the better response of Elspar patients was unlikely to be due to chronological difference in treatment. Standard risk patients made up only 31% (4/13) of Erwinase-treated patients, compared to 55% (41/75) of Elspar-treated patients (Table 1). The smaller proportion of high-risk patients reflects the greater clearance of lymphoblasts with Elspar, demonstrating its greater efficacy.

Toxicity

Toxicity information was available for 96% of patients (Erwinase: 20/21; Elspar: 87/90). The number of patients experiencing grades 3 to 5 toxicities was similar (20%, 4/20 vs 24%, 21/86). Interestingly,

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Table 1. Patient Characteristics and Risk Stratification by L-asparaginase Formulation

	Erwinase (n = 21)	Elspar (n = 89)	Overall (n = 110)
Age (y)	0.67-7.86 Median = 3.66	0.36-13.83 Median = 4.09	0.36-13.83 Median = 3.88
Presenting TWC x10 ⁹ /L	1.08-729 Median = 10.6	1.20-447 Median = 11.7	1.08-447 Median = 11.5
NCI criteria	(n = 21)	(n = 89)	(n = 110)
Low risk	71% (15)	71% (63)	71% (78)
High risk	29% (6)	29% (26)	29% (32)
MRD	(n = 13)	(n = 75)	(n = 88)
Standard risk	31% (4)	55% (41)	44% (45)
Intermediate risk	38% (5)	37% (28)	30% (33)
High risk	31% (4)	8% (6)	9% (10)

MRD: minimal residual disease; NCI: National Cancer Institute

Table 2. Toxicity During Induction

Adverse effect (n= ?)	Erwinase (n = 20)	Elspar (n = 86)
Overall grade 3-5	20% (4)	24% (21)
Overall grade 4	0%	8% (7)
≥Grade 3 infection	20% (4)	12% (10)
≥Grade 3 hepatotoxicity	0%	6% (5)
Diabetes mellitus	0%	3% (3)
Coagulopathy (e.g. thrombosis, stroke, seizure)	0%	7% (6)
Induction deaths	5% (1)- Infection 2% (2)-	Infection, GI bleeding

GI: gastrointestinal

all 4 cases of severe toxicity in Erwinase patients were infection-related, with none experiencing asparaginase-related effects. On the other hand, Elspar patients had a similar rate of infections (12%, 10/86) but, in addition, there was a 3% to 8% incidence of severe hepatotoxicity, diabetes secondary to pancreatitis, coagulopathy and neuropathy (Table 2). There were no asparaginase-related deaths in Erwinase patients but 1 Elspar patient died of gastrointestinal bleeding.

Discussion

This is the first study that utilises MRD to *directly* compare the efficacy of *E. coli*-asparaginase with *Erwinia*-asparaginase. MRD measures the effectiveness of therapy *in vivo*, and patients on Erwinase were 5 times more likely to be stratified into high risk at the end of induction, indicating poorer response. As other induction drugs and patient characteristics were comparable, any difference can be attributed to the different formulations of L-asparaginase used.

The lower efficacy of Erwinase, using the current schedule, is borne out by the poorer outcome of Erwinase-treated patients – 5-year EFS of 62% (13/21) versus 75% (18/24) for Elspar. This is consistent with results from a randomised trial showing 6-year EFS of 60% for *Erwinia*-asparaginase versus 73% for *E. coli*-asparaginase.³

However, fewer patients on Erwinase experienced severe asparaginase-related toxicities like thrombosis, hepatitis and diabetes. The lower efficacy and toxicity of Erwinase can be explained by its shorter half-life and lower activity level *in vivo* compared to Elspar.^{1,2}

Conclusion

This study provides evidence by MRD that Elspar is significantly superior to Erwinase in childhood ALL induction. The greater efficacy of Elspar is accompanied by more severe toxicity, but this is balanced by better treatment response and improved outcome. *E. coli*-asparaginase should be considered the first-line of L-asparaginase therapy for childhood ALL, with *Erwinia*-asparaginase reserved for patients allergic to *E. coli*-asparaginase. Furthermore, we have convincingly shown that MRD can be a powerful tool to compare the efficacy of chemotherapeutic agents in remission induction and treatment of childhood ALL. This can provide faster information early in therapy compared to the conventional relapse outcome 4 to 6 years later.

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Best Oral Presentation Award Finalist – Nursing

Determinants of Patient's Willingness Towards Participation in Clinical Drug Trial in a Psychiatric Setting

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Introduction

More than 82% of clinical research trials have been delayed because there are not enough volunteers.¹ Several studies have shown that there are many factors that determine and influence the

decision made by patient.²⁻⁴ When disease strikes, many people seek a cure through experiment drug in clinical trials: patient pursues trial as a primary path to state-of-art care as a hope after exhausting all conventional treatments and remedies.¹ Thus, it is significant to find

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out if patients, particularly psychiatric patients who need long-term medications will resort to clinical drug trial as a hope of treating their illness.

Materials and Methods

Specific Aims

The purpose of this study was to elucidate and analyse factors that determine the patients' willingness towards participation in clinical drug trial in a psychiatric setting. The information will help us to understand the concerns of the patient for participating in a clinical drug trial.

Methods

This study took on a non-experimental descriptive study design. The scales and questionnaires that were administered in the study are drug attitude inventory-10, rating scale for side effects, SF-12 health questionnaires and self-reporting survey. These scales and questionnaires were administered to 100 patients in a psychiatrist hospital setting as outpatients. Socio-demographic factors, attitudes towards current medication, presence or absence of side-effects, general health of the patient and the self-reporting survey were evaluated. These factors were analysed to see if there was any correlation to willingness in fostering patient's participation in a clinical drug trial.

Statistical Analysis

All analyses were performed using Statistical Package for Social Science 11.5. Statistical significance was set at $P < 0.05$.

Results

Socio-Demographics

A total of 100 patients were recruited in the study. Table 1 shows the demographic details of the patients. Overall, there was no strong interest in participating in the clinical drug trial in any of the demographic groups shown. Out of the 46 participants who expressed interest in clinical drug trial, only 7 (35%) had previously been involved in clinical drug trial. Out of the 54 participants who were not interested in clinical drug trial, 13 (65%) of the participants had participated in a clinical drug trial before.

Scales/Questionnaires

Performing a factor analysis on the 13 self-reported questionnaires, 5 factors were obtained (explaining 69.17% of the variance): benefit factor, trial-related factor, medical factor, satisfaction factor and future treatment and interpersonal factor.

Univariate analysis showed that RSSE, benefit, trial-related and, future treatment and interpersonal factors influenced participation. Those whose scores ranged from 4 to 10 in the rating for side effects were 5.5 (95% CI, 1.10-27.0) times more likely to participate compared with those whose scores ranging from 0 to 3 ($P = 0.041$, Table 2). The median rating was also significantly higher (means that the participant has higher side effects) for those who had interest in participating (Table 2).

Those who scored a higher level of agreement that their interest in clinical drug trial participation was due to benefit, future treatment and interpersonal factors were more likely to participate in a trial ($P < 0.001$).

A logistic regression was performed with all the socio-demographic variables and the scales/questionnaires. The following significant predictors for participation were obtained: Chinese were more likely to be interested to participate compared to non-Chinese (OR = 67.9; 95% CI, 1.4 to 3296; $P = 0.033$). Those whose duration of illness was 1 to 6 years were more likely to be interested to participate compared to those whose illness were more than duration of 13 years (OR = 12831; 95% CI, 11.1-15000; $P = 0.009$). Those who took <3

Table 1. Demographic Results of Patients' Interest in Clinical Drug Trial Participation

	Interest in clinical drug trial participation		P	No. of participants n = 100
	Yes (n = 46)	No (n = 54)		
Age (y)				
18-28	7 (50.0%)	7 (50.0%)	0.982	14
29-39	11 (42.3%)	15 (57.7%)		26
40-50	18 (46.2%)	21 (53.8%)		39
51-61	9 (50.0%)	9 (50.0%)		18
≥62	1 (33.3%)	2 (66.7%)		3
Gender				
Male	20 (55.6%)	16 (44.4%)	0.15	36
Female	26 (40.6%)	38 (59.4%)		64
Marital status				
Single	33 (47.8%)	36 (52.2%)	0.906	69
Married	13 (43.3%)	17 (56.7%)		30
Divorced	0 (0%)	1 (100%)		1
Race				
Chinese	41 (48.2%)	44 (51.8%)	0.091	85
Malay	4 (66.7%)	2 (33.3%)		6
Indian	1 (12.5%)	7 (87.5%)		8
Others	0 (0%)	1 (100%)		1
Religion				
Christianity	15 (38.5%)	24 (61.5%)	0.222	39
Buddhism	15 (60.0%)	10 (40.0%)		25
Hinduism	1 (20.0%)	4 (80.0%)		5
Islam	4 (57.1%)	3 (42.9%)		7
Taoism	4 (30.8%)	9 (69.2%)		13
Others	7 (63.6%)	4 (36.4%)		11
Education level				
No education	2 (66.7%)	1 (33.3%)	0.640	3
Primary	8 (53.3%)	7 (46.7%)		15
Secondary	23 (47.9%)	25 (52.1%)		48
Tertiary	13 (38.2%)	21 (61.8%)		34
Household income				
Below S\$500	14 (48.3%)	15 (51.7%)	0.985	29
S\$501-S\$1000	12 (48.0%)	13 (52.0%)		25
S\$1001-S\$2000	13 (43.3%)	17 (56.7%)		30
S\$2001-S\$3500	3 (50.0%)	3 (50.0%)		6
≥S\$3501	4 (40.0%)	6 (60.0%)		10
Duration of illness (y)				
1-6	18 (64.3%)	10 (35.7%)	0.067	28
7-12	13 (41.9%)	18 (58.1%)		31
≥13	15 (36.6%)	26 (63.4%)		41
Previously involved in any clinical drug trial				
Yes	7 (35.0%)	13 (65.0%)	0.27	20
No	39 (48.8%)	41 (51.3%)		80
Number of medications				
≤2	24 (48.0%)	26 (52.0%)	0.688	50
≥3	22 (44.0%)	28 (56.0%)		50
Number of hospitalisation				
No hospitalisation	5 (45.5%)	6 (54.5%)	0.681	11
1-3 times	21 (42.0%)	29 (58.0%)		50
4-6 times	11 (45.8%)	13 (54.2%)		24
≥7 times	9 (60.0%)	6 (40.0%)		15

medications were more likely to be interested to participate compared to those who took ≥3 medications (OR = 34.9; 95% CI, 2.0-614.9; $P = 0.015$). Those whose side effects rating scores ranged from 4 to 10 were more likely to be interested to participate compared to those whose scores ranged from 0 to 3 (OR = 3347.3; 95% CI, 6.6-5000; $P = 0.011$). Those who scored higher level of agreement that their interest in trial participation was due to future and interpersonal factors were more likely to have interest in the participation (OR = 17.2; 95% CI, 2.7-111.2; $P = 0.003$).

Discussion

The results showed that only 46% of the participants expressed interest in participating in a clinical drug trial. Those who scored higher rating for side effects and those who had illness between 1 and

Table 2. The Scale and Questionnaire Results of the Patient's Interest in Clinical Drug Trial Participant

	Interest in clinical drug trial participation		P
	Yes (n = 46)	No (n = 54)	
Rating for side effects			
0-3	38 (42.2%)	52 (57.8%)	0.041
4-10	8 (80.0%)	2 (20.0%)	
Rating for side effects			
Mean (SD)	1.52 (2.12)	0.57 (1.21)	0.005
Range	0 to 10	0 to 6	
Median	1.0	0	
Drug Attitude Inventory (DAI)			
Non-compliant	5 (45.5%)	6 (54.5%)	0.974
Compliant	40 (46.0%)	47 (54.0%)	
SF12 (QOL)			
Physical functioning			
Mean (SD)	5.0 (1.2)	4.9 (1.2)	0.516
Range	2.0 to 6.0	2.0 to 6.0	
Median	6.0	5.0	
Role physical			
Mean (SD)	3.7 (0.7)	3.5 (0.9)	0.169
Range	2.0 to 4.0	2.0 to 4.0	
Median	4.0	4.0	
Role emotional			
Mean (SD)	3.7 (0.7)	3.5 (0.9)	0.299
Range	2.0 to 4.0	2.0 to 4.0	
Median	4.0	4.0	
General health			
Excellent	0 (0%)	2 (100%)	0.365
Very Good	11 (40.7%)	16 (59.3%)	
Good	21 (44.7%)	26 (55.3%)	
Fair	12 (54.5%)	10 (45.5%)	
Poor	2 (100%)	0 (0%)	

6 years were more likely to participate in the clinical drug trial. Thus, the degree or severity of the side effects on the patients' current medication and the stage of their mental illness such as those who are newly diagnosed or not in the chronic stage contribute and therefore, determine their interest in clinical drug trial participation.

In performing factor analysis on the self-reporting survey, high scores on trial-related and, future treatment and interpersonal are factors for encouraging participation in clinical drug trial.

Conclusion

This study showed that very few patients are interested in clinical drug trials. The findings of the study showed significant differences on the factors, which can be explored, in a greater depth such as the trial-related and interpersonal factors. The information that we have elucidated from the study will help us to further assess patient's attitude and to understand patient's reasons for not participating in a clinical drug trial. The personnel who will be conducting the trial perhaps can address this.

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Best Oral Presentation Award Finalist – Nursing

Algorithm to Achieve Prescribed Dialysis Adequacy Targets for Non-compliant Children on Automated Peritoneal Dialysis (APD)

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Introduction

Automated peritoneal dialysis (APD) is the dialysis modality of choice in children with end stage renal disease (ESRD).¹ It is thus important to optimise APD prescriptions in young patients in order to achieve dialysis adequacy targets and in turn, improve clinical outcome. There is evidence that higher total weekly urea clearance (Kt/Vurea) and creatinine clearance (CCr) values are associated with lower morbidity and mortality rates.² Hence, it is current clinical practice to use computerized kinetic modeling of Kt/Vurea and CCr as a tool for modifying APD prescriptions, in order to achieve such dialysis adequacy targets.^{3,4}

However, non-compliance is a major factor in failing to achieve dialysis adequacy targets, particularly in adolescents.^{5,6} To compensate for shortfalls due to non-compliance, clinicians often advise non-compliant patients to carry out 'extra dialysis' on non-working days by increasing the number of hours or cycles of dialysis or the dialysate glucose concentration. The clinical outcomes of such 'compensatory dialysis' has never been studied. The study aimed firstly at designing a technique to compute dialysis adequacy in the non-compliant patient, and subsequently to develop an algorithm to calculate the daily prescription adjustments required to compensate for any shortfalls in preceding days,

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such that dialysis adequacy targets are met even in the non-compliant patient.

Materials and Methods

A total of 14 patients undergoing APD, who are currently on chronic peritoneal dialysis in the pediatric renal replacement program at the Shaw-NKF Children's Kidney Centre in Singapore, were recruited into the study on a voluntarily basis. The patients were from 3 to 25 years of age and were on APD for a mean of 2.7 ± 2.4 (range, 0.5 to 8.1) years. No patients had peritonitis during the study or 1 year prior to entering the study.

Using predicted Kt/Vurea and CCr data generated by PD Adequest® 2.0 program, secondary models were derived by multivariate regression as functions of the prescription parameters (coefficient of determination $r^2 > 0.99$). An algorithm was formulated, based on these secondary models, to compute the monthly average of the daily Kt/Vurea and CCr values (i.e. computed Kt/Vurea and CCr). The study comprised 3 phases of 1 month duration each. In phase I, patients were compliant with the APD prescription. In phase II, patients were non-compliant for at least 3 days per week. In phase III, an adjustment program was written for the APD prescription so that daily changes could be made to compensate for shortfalls due to non-compliance. Data from the APD cyclers were recorded on the HomeChoice PRO™ System.

Results

In phase I (compliant phase), the algorithm-computed Kt/Vurea and CCr, had excellent agreement with the Adequest-predicted parameters (intraclass correlation $r_i = 0.99$, Bland-Altman coefficient of clinical agreement $C_{CA} = 98\%$). In phase II (non-compliant phase), the algorithm-computed Kt/Vurea (1.7 ± 0.5) and CCr (42.4 ± 15.6) were significantly lower than Adequest-predicted Kt/Vurea (1.8 ± 0.6) and CCr parameters (44.4 ± 16.9) ($P = 0.001$). In phase III, using the daily adjustment program to compensate for shortfalls, both the algorithm-computed Kt/Vurea (Fig. 1) and CCr (Fig. 2) had good agreement with the Adequest-predicted parameters ($r_i = 0.98$ and 0.99 respectively and $C_{CA} = 100\%$ for both).

Discussion

It was firstly important to validate the use of PD ADEQUEST 2.0 in our population. In phase I of our study, the ADEQUEST-predicted Kt/Vurea and CCr values correlated well with the actual measured values, with an intraclass correlation coefficient of 0.72 and 0.77 respectively. Our results in pediatric patients were similar to that reported by Warady et al.,⁴ where the concordance correlation between ADEQUEST-predicted and measured results were 0.70 for total weekly Kt/Vurea and 0.77 for total weekly CCr. Hence, PD ADEQUEST 2.0 accurately predicts total Kt/Vurea and CCr adequacy targets in our sample population. In addition, the strong correlation between ADEQUEST-predicted and algorithm-computed Kt/Vurea and CCr values in phase I demonstrated that the algorithm had been accurately derived from the predicted values generated by PD ADEQUEST 2.0.

In phase II, patients were non-compliant. The algorithm-computed Kt/Vurea and CCr values in phase II were significantly lower than the ADEQUEST-predicted targets. This demonstrates the algorithm's sensitivity to daily changes and shortfalls in the dialysis regimen due to non-compliance.

In phase III, despite intermittent non-compliance, adjustments to the daily dialysis regimen could be computed by the algorithm. Patients were able to carry out these recommended 'extra dialysis' adjustments such that the computed values, which had been lowered due to non-compliance, could attain the ADEQUEST-predicted

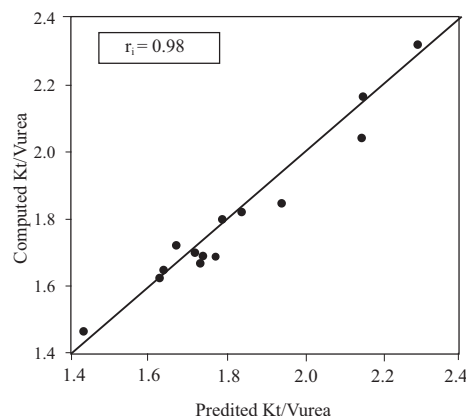


Fig. 1. Plot of Phase III algorithm-computed against ADEQUEST-predicted Kt/Vurea values with intraclass correlation coefficient (r_i).

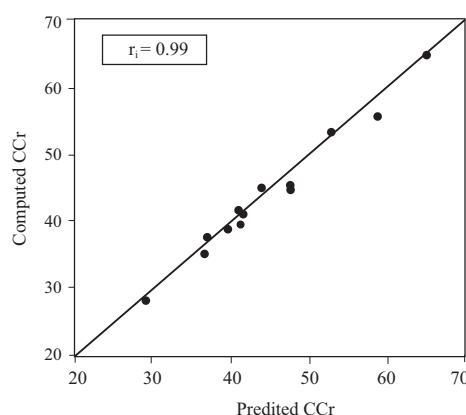


Fig. 2. Plot of Phase III algorithm-computed against ADEQUEST-predicted or CCr values with intraclass correlation coefficient (r_i).

targets. In other words, the patient has been fully compensated for shortfalls. This is clearly shown by the good agreement between the algorithm-computed values and ADEQUEST-predicted targets in this phase.

For all 3 phases, the measured and algorithm-computed Kt/Vurea and CCr values correlated reasonably well. However, the algorithm-computed values are sensitive to changes in the daily dialysis regimen over a certain period of time, while the measured results represent clearance from the previous night's dialysis only. As the 2 methods of measuring adequacy have a different basis, the significance of comparing computed and measured values is questionable.

The concept of using the computed average Kt/Vurea and CCr values instead of actual measured results to assess adequacy in patients is new. Thus, further research is necessary to compare the clinical outcomes of fully compensated non-compliant dialysis patients (using the algorithm) with consistently noncompliant patients and consistently compliant patients. This would determine whether 'compensatory dialysis' can provide adequate clearance of uremic toxins over the long term. The algorithm, which calculates the optimal dialysis dose to fully compensate noncompliant patients, can be used to facilitate such research.

Conclusion

In conclusion, the results presented here show that our algorithm computes adequacy Kt/Vurea and CCr values, taking into consideration changes in the daily regimen over a one month period.

It was also clinically possible to use the algorithm to calculate the necessary 'compensatory' prescription such that predicted target Kt/Vurea and CCr were achievable using the algorithm for adjustment of the daily APD prescription, despite shortfalls due to non-compliance. Further research is necessary to investigate the long-term outcome of non-compliant patients, who are compensated for shortfalls using the algorithm.

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Best Oral Presentation Award Finalist – Nursing

Health Screening Outcomes that Lead to Health Seeking Behaviours

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Introduction

Health screening is an essential component of disease management in the community. There is little controversy that early detection and treatment of risk factors or diseases such as diabetes, hypertension and renal disease, which can save lives and minimize potential complication and unnecessary morbidity.¹

In 1997, the National Kidney Foundation (NKF) launched a nation-wide community health-screening programme. It is a community-based screening programme, which targets detection of diseases such as diabetes, hypertension and high blood cholesterol in asymptomatic people aged 18 and above. These 3 conditions, if poorly managed, could eventually lead to heart disease, stroke and end stage renal failure (ESRF). This programme also includes a nurse-based educational component that emphasizes healthy living and the need for regular screening (referral to the NKF prevention centres and affiliated physicians for recheck of abnormal values).

During the health-screening session, the following procedures are done:

1. Weight and height measurement
2. Random blood glucose and random total cholesterol
3. Urine test (consist of testing any presence of blood, protein, glucose, ketones, and pH)
4. Waist-hip ratio measurement
5. Blood pressure measurement
6. Counselling (providing advice on participants' abnormalities)

To date, more than 1.1 million screening episodes have been conducted island-wide; few studies have examined whether participants with screen-detected abnormalities attend confirmatory evaluation.^{2,3} We sought to determine the health-seeking behaviour among health-screening participants with abnormal test results, and compare the characteristics of participants who sought and did not seek confirmatory evaluation. We also attempted to identify potential risk factors for inappropriate behavior.

Materials and Methods

A sample of health screening attendees with raised blood glucose level (≥ 140 mg/dL), total blood cholesterol level (≥ 200 mg/dL),

blood pressure reading ($\geq 140/90$ mm Hg), proteinuria and haematuria screened during the period from June 2003 to March 2004 were identified from our patient database (sample size = 1255). Demographic data of the attendees were collected for age, ethnic group, gender and educational status. Nurses conducted standardised follow-up telephone interviews with the respondents. Each respondent was asked whether they had gone for confirmatory tests with a physician pertaining to the screening abnormality that was detected by the nurses at the health screening session.

A maximum of 2 calls were made before a subject was excluded from the study. Respondents were grouped according to whether they had gone for confirmatory evaluation. Demographic characteristics were compared among the 2 groups. The Chi-square test was used for statistical analysis.

Results

One thousand, one hundred and forty-seven participants, with complete data, were included in the study. The response rate was 49.5% (568 respondents). Five hundred and seventy-nine participants (non-respondents) could not be contacted. However, demographic characteristics such as ethnicity, gender, age and educational status among respondents and non-respondents were similar. Two hundred and seventy-eight (48%) of the 568 respondents had visited a physician to do confirmatory tests, after being told to recheck their abnormal results.

Respondents who did not seek confirmatory evaluations had higher educational status compared to those who did ($P = 0.005$). There was no difference in gender, age, and ethnicity among respondents who attended and those who did not attend confirmatory evaluation with their physicians (Table 1). Respondents who have raised blood glucose (60.9%), raised blood pressure (60.2%) and haematuria (60.2%) were more likely to seek confirmatory tests (Table 2).

Discussion

The appropriate health-seeking behaviour of seeing a doctor for confirmation and management of screen-detected abnormalities in our participants were relatively high (48.9%, 278/568). In comparison

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Table 1

Characteristics	Consulted doctor (%)	Did not consult doctor (%)	P
Gender			
Male	139 (46.6%)	159 (53.4%)	0.249
Female	139 (51.5%)	131 (48.5%)	
Age (y)			
18-39	166 (49.8%)	167 (50.2%)	0.123
40-59	83 (44.4%)	104 (55.6%)	
≥60	29 (60.4%)	19 (39.6%)	
Race			
Chinese	252 (49.8%)	254 (50.2%)	0.106
Malay	10 (29.4%)	24 (70.6%)	
Indian	13 (56.5%)	10 (43.5%)	
Others	3 (60%)	2 (40%)	
Language			
English	182 (48.7%)	192 (51.3%)	0.799
Chinese	87 (50.3%)	86 (49.7%)	
English & Chinese	9 (42.9%)	12 (57.1%)	

Table 2

Characteristics	Consulted doctor (%)	Did not consult doctor (%)	P
Abnormalities			
Blood glucose	78 (60.9%)	50 (39.1%)	0.000
Blood pressure	56 (60.2%)	37 (39.8%)	
Blood in the urine	59 (60.2%)	39 (39.8%)	
Cholesterol	46 (37.4%)	77 (62.6%)	
Protein in the urine	39 (31.0%)	87 (69.0%)	
Education			
Below tertiary	181 (53.9%)	155 (46.1%)	0.05
Tertiary and above	97 (41.8%)	135 (58.2%)	

to the “Check Your Health” Community Health Screening Programme, only 15.4% (1530/9934) of the participants have attended a follow-up recheck with a physician.⁴ The higher adherence rate may be due to the sufficient time given over the counselling station for each participant, which results in a better understanding of the

importance of follow-up actions with a physician in regards to their abnormalities.

Raised blood glucose, raised blood pressure and haematuria were the abnormalities most likely to result in consultation for our participants. Fontana et al⁵ have also reported increased consultations with physicians for patients with detected raised blood pressure during their primary health screening. Higher educated people are also less likely to seek confirmatory evaluation.

As treatment for diabetes, hypertension and high blood cholesterol level is crucial to prevent related complications such as end-stage renal disease, the NKF sends follow-up letters to those with abnormal results to remind them to seek further confirmatory tests with their doctors. As a result of the survey, the foundation has also commenced telephone follow-ups to reinforce the need for future evaluation. Its 2 prevention centres have also expanded their services to help participants confirm any abnormalities.

Conclusion

Health screening is a vital component of healthy living. Follow-up action is essential to ensure that participants with screened detected abnormality seek appropriate medical advice.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Clinical Based) – Ethicon Surgical Book Prize (Clinical)

Long-term Survival Following Liver Resection for Colorectal Metastases – An Adelaide Experience

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Introduction

The aim of this study was to assess patient outcomes as well as the factors influencing patient and disease free survival in a cohort of patients treated over the last decade for surgically resectable liver metastases from colorectal primaries. Kaplan-Meier survival statistics and Cox regression were used to analyse factors that affected survival.

Materials and Methods

This was a retrospective study of prospectively collected data over the last 10 years, from February 1992 till April 2003. Data were taken from patients' operation reports and charts, and also direct follow-up with the respective general practitioners, referring physicians, oncologists and surgeons. Mortality was double checked with the cancer registry. The last follow-up death recorded was 21 April 2003.

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All cases were assessed by CT and MRI prior to surgery, with the use of routine PET scan in the past 3 years to exclude extra hepatic disease. It was the unit's policy to apply minimal resection to achieve tumour clearance. Routine intraoperative ultrasound was used. Parenchymal transection was achieved via a variety of techniques, more recently with CUSA and harmonic shears, with the pedicles being stapled.

Results

All patients who were operated in this centre were entered into the study. A total of 72 patients were treated over the period from February 1992 till April 2003. There were 53 male and 19 female patients. The median age was 63.8 (range, 31.7 to 81.7) years, with a mean of 63.2 years. The median follow-up period was 3.5 (range, 0.1 to 11.2) years, with a mean of 4.22 years. At the end of the study, there were 27 deaths and 45 patients who were alive, with 29 being disease free. Clear margins in this study were defined as those with margins microscopically free of tumour.

The results of the pathological resection margins, original colonic staging and dominant metastatic size are shown below:

- Resection margin
 - Involved (or <1 mm) 4 (5%)
 - <2 mm 7 (10%)
 - Clear margin 61 (85%)
- Colonic staging
 - A = 5 (7.7%)
 - B = 21 (32.3%)
 - C = 39 (60%)
 - D = 5 (7.7%)
- Dominant metastasis size
 - Median 45 mm (range, 6 to 140 mm)
 - Mean 49 mm

Complications

There were no operative or hospital mortality. There were 7 bile leaks, 4 of which needed percutaneous drainage, with the leak sealing off. Two needed ERCP stenting, also with subsequent sealing of the leak and stent removal.

There were 7 chest infections, 1 prolonged small bowel ileus, which resolved with conservative management, and 1 patient who had a haematoma around the surgical site, which was drained percutaneously.

The Kaplan Meier curve in Figure 1 illustrates the overall patient survival and the patient disease free survival over a period of 5 years. The institution achieved a 1-year patient survival rate of 95% and a

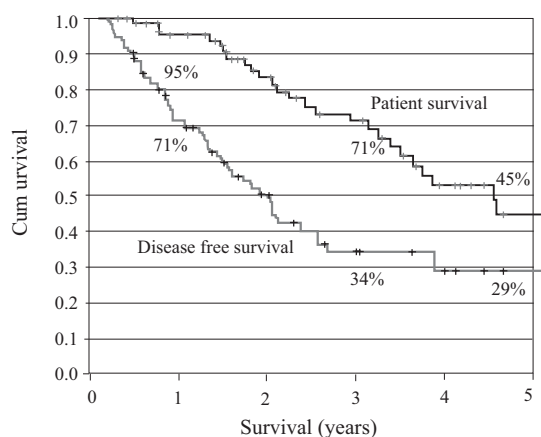


Fig. 1. Patient vs disease free survival.

5-year survival rate of 45%. The disease free survival rate was 71% in 1 year and 29% in 5 years.

A univariate analysis of various determinants which may affect patient overall survival revealed that age >70 years was significant with a *P* value of <0.002, and tumours >3 cm approached significance with a *P* value of 0.055. Sex, the type of resection, location of disease, Duke's staging of the colorectal tumour, the number of tumours, the resection margins, chemotherapy and the interval between colonic and liver resection were not significant.

With regards to disease free survival, age >70 years and bilobar disease proved significant in affecting recurrence of disease, with a *P* value of <0.004 and <0.01, respectively. Sex, the type of resection, Duke's staging of the colorectal tumour, the number of tumours, the resection margins, chemotherapy and the interval between colonic and liver resection were not significant in affecting recurrence.

Outcomes

Of the 72 patients treated, 45 patients were alive at the end of the study, with 29 among them disease free. Of the 43 who had recurrence of the disease, 26 (67%) had disease which included the liver, with 15 (38%) who had only liver recurrence. Fourteen had disease which included the lung (36%) and 3 had recurrent disease in other sites in the abdomen. Thirteen (33%) had disease recur in more than 1 site.

Five-year Patients' Survival

Author	Institution	Year	No.	5-year survival (%)
Foster	Multi	1978	170	20
Adson	Mayo Clinic	1984	141	25
Hughes	Multi	1988	859	33
Iwatsuki	Pittsburg/Colorado	1988	90	36
Fong	MSKCC	1997	456	38
Choti	John Hopkins	2002	226	40
Current series	Flinders + Ashford Med Centres	2003	72	45

Five-year Disease-free Survival

Author	Institution	Year	Number	5-year survival (%)
Harmon	Virginia Mason	1999	110	28
Choti	John Hopkins	2002	226	20
Kokudo	Cancer Institute Hospital, Tokyo	2002	102	29.7
Current series	Flinders + Ashford Med Centre	2003	72	29

Our institution had very comparable rates to current reported standards, with a 45% overall 5-year survival and a 29% 5-year disease free survival.

Conclusion

The patient 3- and 5-year survival after curative resection for colorectal metastasis was 71% and 45%, respectively. Twenty-nine per cent survived without recurrence at 5 years. Ninety-five per cent of cases had clear margins. Patient's age >70 years is associated with poorer patient survival, and metastatic size >3cm has shown a trend towards poorer survival, with a *P* value of 0.055. Anatomical resection in this study did not confer any advantage over non-anatomical resection. Bilobar disease was significant in affecting the recurrence of disease.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Clinical Based) – Ethicon Surgical Book Prize (Clinical)

Rapid Prenatal Diagnosis by AmnioPCR and AmnioFISH: Routine Testing for Down's Syndrome (Trisomy 21) and Sex Chromosome Trisomies, but Targeted Testing for Edward's (Trisomy 18) and Patau's Syndromes (Trisomy 13)

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Introduction

Prenatal diagnosis of chromosomal abnormality using conventional metaphase karyotyping requires up to three weeks for the full diagnosis to be released. This delay results in significant parental anxiety. Amniotic fluid polymerase chain reaction (Amnio-PCR) and fluorescence *in situ* hybridisation (Amnio-FISH) are new, but expensive, molecular genetic techniques allowing rapid prenatal detection of fetal aneuploidy within 24 to 72 hours. Amnio-PCR involves identification and amplification of small-tandem-repeat (STR) markers located on the specific chromosome to identify the presence or absence of a chromosome. In Amnio-FISH, a chromosome is recognised by being labelled with a fluorescence probe. The efficacy of these techniques and the reduction of parental anxiety have been well-documented.¹⁻³ Given a choice, most patients and healthcare providers would request rapid tests followed by a full conventional karyotype.⁴ The main limitation of these tests is the cost incurred by the individual and the healthcare system. The challenge is to find an economical way to provide these tests for a broader population.

Aneuploidies involving chromosomes 13, 18, 21, X and Y account for more than 80% of significant chromosomal abnormalities.³ Therefore, prenatal samples are routinely analysed by PCR or FISH for all these 5 chromosomes in most centres, while awaiting the karyotype. Few fetuses with trisomy 13 or 18 have no ultrasound abnormalities.³ We hypothesised that if we test for trisomy 21 and sex chromosome trisomies routinely, but for trisomy 13 or 18 only if ultrasound abnormalities were present, we would rapidly detect more than 90% of these fetal aneuploidies.

Materials and Methods

From January 1992 to April 2004, 14,091 antenatal diagnostic procedures were performed in our department, out of which 262 cases of trisomy 13, 18, 21 and sex chromosome abnormalities were diagnosed. The referral criteria for prenatal karyotyping included advanced maternal age, positive screening tests, abnormal ultrasonographic findings, previous child with chromosomal abnormality and parental chromosomal rearrangement.

The data were obtained via our electronic database and clinical notes. The presence or absence of ultrasound abnormalities was

analysed for each case. The number and the type of abnormalities were also reviewed for each patient. The ultrasonographic and Amnio-FISH features of fetuses with chromosomal aneuploidies are shown in Figure 1.

Kruskal-Wallis and Mann-Whitney tests were used for analysis of these data.

Results

Distinct ultrasonographic abnormalities were seen in 76.7% of trisomy 18 (n = 46) and 90.0% of trisomy 13 (n = 18) fetuses. Only 23.3% of trisomy 18 (n = 14) and 10.0% of trisomy 13 (n = 2) fetuses had no detectable ultrasound abnormalities. In contrast, only 58.2% of trisomy 21 fetuses (n = 71) had structural abnormalities, whereas 41.8% of them (n = 51) had no detectable ultrasound abnormalities.

For sex chromosome trisomies (XXX, XXY, XYY), only 6.5% (n = 2) had structural abnormalities, but 96.6% (n = 28) monosomy X fetuses had abnormalities. The ultrasound evaluation of 93.5% (n = 29) of fetuses with sex chromosome trisomies and 3.4% (n = 1) of monosomy X fetuses were normal. However, as an entity, 50% of these fetuses with sex chromosome abnormalities had no ultrasonographic abnormalities.

This difference in the presence or absence of structural abnormalities in these cases of fetal aneuploidies is statistically significant ($\chi^2 = 68.5$; df = 4; $P < 0.001$). If rapid detection for trisomy 21 and sex chromosome abnormalities were performed routinely, whereas Amnio-PCR /Amnio-FISH for trisomy 13 and 18 were performed only if indicated by the presence of abnormalities, 93.9% of these aneuploidies would be identified ($z = 3.2$; $P = 0.001$) (Fig. 2).

Our data showed that routine testing for trisomy 21 is recommended, as a significant percentage of abnormal fetuses would otherwise be missed with the rapid tests. However, for trisomy 13 and 18, the majority of the fetuses have ultrasound abnormalities. In fact, the majority of these fetuses have multiple rather than single abnormalities. It would therefore be more cost-effective if Amnio-PCR/Amnio-FISH were done for chromosomes 13 and 18 only if indicated by abnormal ultrasound findings.

For sex chromosome abnormalities, our study showed clearly that most of the fetuses with sex chromosome trisomies have no structural

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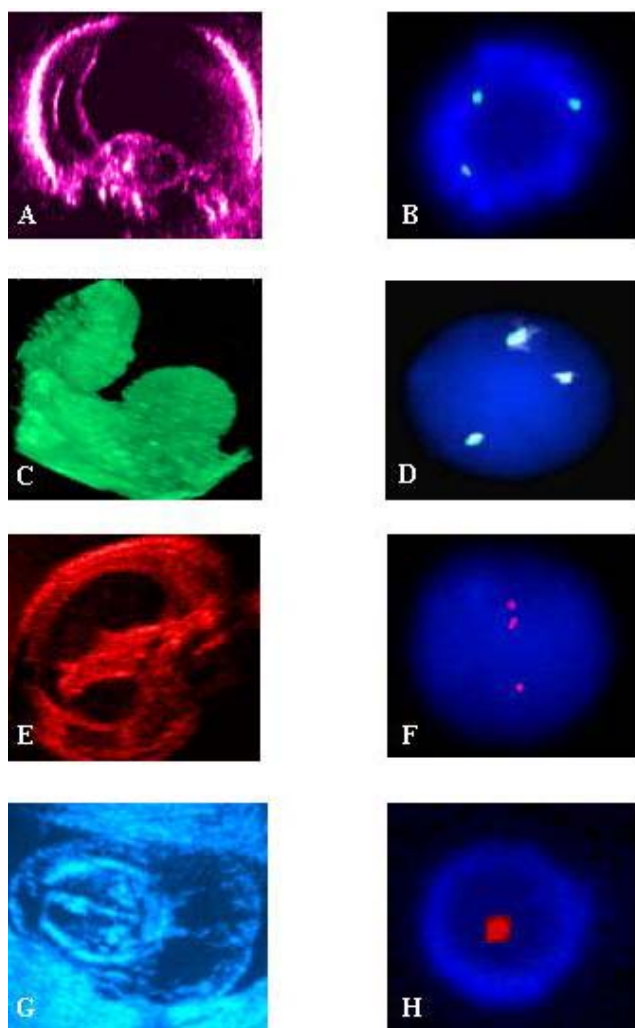


Fig. 1. Ultrasongraphic and Amnio-FISH features of fetuses with chromosomal aneuploidies. (A) Holoprosencephaly in Trisomy 13. (B) Amnio-FISH showed 3 copies of chromosome 13. (C) Exomphalos in Trisomy 18. (D) Amnio-FISH revealed 3 copies of chromosome 18. (E) Double bubble sign characteristic of duodenal atresia in Trisomy 21. (F) Amnio-FISH showed 3 copies of chromosome 21. (G) Cystic hygroma is a characteristic feature of Turner's syndrome (XO). (H) Amnio-FISH showed a single copy of chromosome X.

abnormalities. However, almost all the monosomy X fetuses have distinct ultrasongraphic abnormalities. The analysis of sex chromosome abnormalities as an entity suggested that rapid testing for chromosomes X and Y is still recommended as half of them appear ultrasongraphically normal.

Discussion

We have shown that a significant proportion of fetuses with trisomy 21 and sex chromosome abnormalities had no structural abnormalities but most of trisomy 13 and 18 fetuses have ultrasongraphic abnormalities. The findings suggest that routine testing should be done for chromosomes 21, X and Y, but targeted testing can be performed for chromosomes 13 and 18, when prenatal samples are sent for Amnio-PCR/Amnio-FISH.

As prenatal ultrasound technology advances, especially with the introduction of 3 or 4 dimensional ultrasound machines, the sensitivity

Abnormality	Detected	Missed
	Routine testing 21, X and Y 100%	
	Targeted testing 13 and 18 80.0%	
	Combined Strategy 93.9%	6.1%

Fig. 2. Routine rapid testing for chromosomes 21, X and Y (100% detection rate) and targeted testing for chromosomes 13 and 18 (80% detection rate) allows almost 94% rapid detection of these aneuploidies ($z = 3.2$; $P = 0.001$).

of detecting abnormalities will continue to improve. This will further reduce the number of cases where abnormalities are missed on ultrasound evaluation. It will therefore make the strategy of performing rapid tests based on ultrasound indications even more accurate.

If the cost issue can be alleviated, the healthcare providers will be able to provide rapid results for a broader patient population. Implementation of this service could lead to rapid diagnosis of abnormalities and early reassurance for women with normal results. Earlier clinical decision could also be made for patients with abnormal results. The American College of Medical Genetics and most authors agreed that clinical decision can be made if the positive PCR/FISH results are associated with ultrasound findings compatible with the aneuploidy detected by the rapid prenatal tests.⁴⁻⁶ Evans et al⁶ had shown in a series of over 300 high-risk patients that there was 100% concordance between ultrasongraphic predictions of aneuploidy, and confirmation with FISH results.

Our work showed that, when performing Amnio-PCR/Amnio-FISH, routine testing for trisomy 21 and sex chromosome abnormalities but targeted testing for trisomies 13 and 18 based upon ultrasound evidence of abnormality is scientifically sound. Costing less, and allowing rapid detection of almost 94% of significant fetal aneuploidies, this novel approach will be readily accepted by couples-at-risk. This strategy can therefore allow rapid prenatal tests for a wider, even nationwide population.

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Reconstruction of the Ocular Surface by Transplantation of a Serum free Cultivated Conjunctival Tissue equivalent

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Introduction

Damage to the conjunctiva may arise from various diseases, such as pterygia, conjunctival tumours, chemical injury or surgical trauma. Treatment of these disorders, by surgical removal of large areas of conjunctiva results in subconjunctival scarring and fibrosis, and may be complicated by granuloma formation, cicatricial fornix shortening, symblepharon formation, or ocular motility restriction.¹ The use of free conjunctival autografts to repair these surgical defects is accompanied by iatrogenic injury to the donor site, and may further complicate the management of patients with extensive or bilateral ocular surface disorders.

The use of bioengineered conjunctival equivalents represents a novel approach to replace conjunctiva, without causing iatrogenic injury associated with the harvesting of large autografts. It is particularly useful in situations where the normal conjunctiva is deficient either from disease or scarring. Conventional culture methods for ocular surface epithelial cells require the use of bovine serum and murine 3T3 feeder cells, with the attendant risks of transmission of zoonotic infection (e.g. bovine spongiform encephalitis) and xenograft rejection.²⁻⁵ The use of serum-free media is therefore significantly advantageous, as it eliminates the need for serum and feeder cells, thereby reducing these risks.

We describe the development of serum-free derived autologous conjunctival tissue-equivalents and report the novel use of these cultivated conjunctival equivalents for ocular surface transplantation and reconstruction.

Materials and Methods

We conducted a prospective clinical trial involving autologous cultivated conjunctival transplantation for the treatment of ocular surface diseases. This study was approved by the Institutional Review Board and written informed consent was obtained for all patients.

Development of a Conjunctival Tissue-Equivalent

Preparation of human amniotic membrane (HAM) substrates. HAMs were obtained from healthy mothers who had undergone cesarean sections. The HAMs were cleaned and placed basement-membrane side up on a nitrocellulose support. They were then incubated with Dispase II 1.2 U/mL to remove any amniotic epithelial cells.

Ex vivo expansion of conjunctival epithelial cells. Superior forniceal conjunctival biopsies were obtained from healthy donors undergoing routine pterygium or cataract surgery. The conjunctival tissues were cut into 0.5 mm pieces, and cultivated on the HAM under serum-free conditions, at 37°C, 5% CO₂ and 95% air, with media change carried out every 2 days. Upon reaching confluence, cells were exposed to differentiating medium to promote stratification. Two weeks later,

a confluent stratified epithelial sheet was obtained over the HAM substrate.

The conjunctival equivalents were analysed by light and electron microscopy. The expression of keratins K4 and 19, and MUC5AC goblet cell mucin, was determined by immunohistochemistry.

Ocular Surface Transplantation

All patients underwent a superior forniceal conjunctival biopsy, measuring 1 mm x 3 mm in size. The conjunctival epithelium was carefully dissected free from the underlying tenons, cut into 0.5 mm pieces, and inoculated onto the basement-membrane side of the HAM in serum-free media, using the methods described above.

Transplantation of cultivated conjunctival equivalents. Definitive surgery 2 weeks later involved excision of the diseased conjunctiva and transplantation of the conjunctival equivalent. The area of diseased conjunctiva was first excised. The conjunctival equivalent was then cut in size to match the surgical defect and sutured in place with interrupted 10/0 vicryl sutures. Tobradex eyedrops were administered for 1 month following surgery.

Postoperatively, these patients were monitored with serial slit-lamp examinations and fluorescein staining to access the epithelial integrity. Main outcome measures included maintenance of conjunctival epithelialisation, integrity of the graft, resolution of the disease, and presence of complications. All patients were followed up for a minimum of 6 months.

Results

Twenty-six patients with various ocular surface diseases requiring conjunctival transplantation and reconstruction were treated. The mean age of patients was 48.3 ± 12.1 years (range, 9 to 72 years). There were 13 males and 13 females. Fourteen left eyes and 12 right eyes were operated on. The mean follow-up period was 12.9 ± 4.3 months (range, 8 to 18 months).

A stratified conjunctival epithelial sheet was formed on the HAMs. Following transplantation, complete epithelialisation was confirmed by the absence of fluorescein staining within 72 hours. Patients were noted to have less conjunctival inflammation, as compared to conventional autograft surgery. A good functional and cosmetic result was achieved in all eyes. A successful outcome, as defined as resolution of the disease, maintenance of conjunctival epithelialisation and maintenance of graft integrity was obtained in all patients. There were no cases with significant subconjunctival scarring, symblepharon formation, eyelid cicatrization, or ocular motility restriction. No significant complications were noted during the follow-up period and the ocular surface with surviving transplanted epithelia remained stable and healthy.

Ultrastructural examination of the conjunctival equivalents revealed

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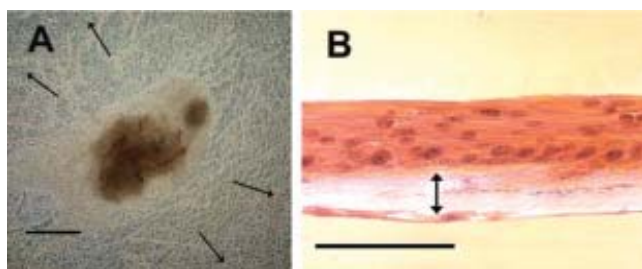


Fig. 1. Human conjunctival epithelial cells cultivated on a HAM substrate. (A), epithelial cell outgrowth from explant culture on day 3, as observed under a phase contrast microscope. (B), light microscopy demonstrating a stratified conjunctival epithelial sheet on HAM (HAM is indicated by the double-headed arrow). (A) Bar = 500 μ m, (B) Bar = 63 μ m.

apical microvilli and a basal lamina with hemidesmosomes. Immunostaining confirmed the expression of cytokeratins 4 and 19, and MUC5AC mucin.

Discussion

We describe the safe and effective use of autologous cultivated conjunctival transplantation for the treatment of ocular surface disease. This treatment modality results in almost immediate epithelialization of the ocular surface, thereby allowing earlier ocular rehabilitation, a reduction in postoperative inflammation, and a rapid return of the protective and supportive function provided by the conjunctiva.

Small conjunctival biopsies were taken from the fornix, the site enriched in conjunctiva stem cells.⁶ A significant advantage is the minimal damage to the normal conjunctival surface, as compared to conventional surgery where large pieces of normal conjunctiva may need to be harvested. The use of autologous tissue minimizes any risk of immunologic rejection and negates the use of immunosuppressive therapy. In addition, the use of amniotic membranes has been shown to promote wound healing and reduce scarring.

Previous studies on epithelial cell culture have required the use of animal serum and feeder cells, with the attendant risks of zoonotic infection and xenograft rejection.²⁻⁵ The elimination of bovine serum and feeder cells from our culture system has the advantage that it reduces the risks associated with the use of animal material for clinical transplantation.

Transplantation of autologous serum-free cultivated conjunctival equivalents may provide a novel method for treating a wide range of

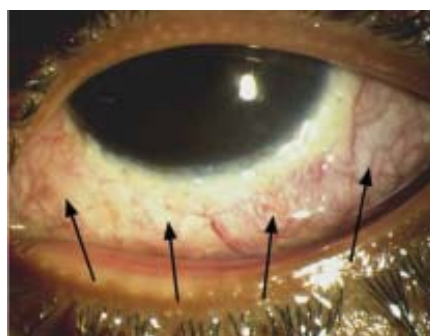


Fig. 2. Day 3 postoperative appearance, demonstrating restoration of the ocular surface following cultivated conjunctival transplantation. The transplanted conjunctival tissue-equivalent shown here has resurfaced the entire inferior bulbar conjunctival surface (indicated by arrows), following a large surgical excision of diseased tissue.

ocular surface disorders where the normal conjunctiva is damaged or deficient. This is particularly useful in diseases requiring extensive conjunctival excision, bilateral diseases, severe ocular surface disease with conjunctival contracture, or when preservation of the conjunctiva is required for future glaucoma surgery. These findings bring us one step closer towards the development of a safe and effective xenobiotic-free bioengineered tissue-equivalent for clinical transplantation.

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The Expression of Insulin-like Growth Factor-I in Periodontal Healing Following Tooth Replantation

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Introduction

Tooth avulsion represents the most severe form of dental injuries and is known to inflict extensive damages to the pulp and periodontium. While necrotic pulp resulting from severance of apical neurovasculature could be dealt with by optimal timely endodontic therapy, it is the re-establishment of the vital intact periodontal ligament (PDL) that is crucial for maintaining a stable tooth/bone interface and essential for tooth retention.

Attempts to regenerate functional periodontium after tooth replantation following undesirable prolonged extra-oral duration/condition, which could potentially lead to tooth loss, had not been met with success. The mechanism underlying functional healing following replantation of avulsed teeth with severely damaged periodontium had not been fully elucidated.¹

Recent evidence has implicated growth factors in the wound repair and regenerative processes. Insulin-like Growth Factor (IGF) has been shown to play biologic roles in periodontal disease model.² Nevertheless, there is no known study to date evaluating the immunohistochemical profiles of IGF-I potentially modulating the periodontal healing following tooth replantation.

Aim

The aim of this study was to characterise the early temporal expression of IGF-I in the normal periodontium and the healing periodontal tissues following immediate and delayed replantation.

Materials and Methods

Experimental Procedures

The animal surgical experiments were performed in accordance with the International Guiding Principles for Animal Research. Under general anaesthesia, periodontally healthy and caries-free mature teeth from 6 adult mongrel dogs (20 kg) were endodontically treated to prevent inflammatory root resorption of pulp origin. The hemisected premolar roots were used as separate units.

Experimental Samples

The experimental samples consisted of 64 randomly distributed roots in the control group and 2 treatment groups. The roots in the non-experimental control group (serving as a baseline) were not extracted. The roots in the immediate replantation (representing optimal healing) and in the delayed-replantation (representing adverse healing) treatment groups were extracted simulating avulsion injury and atraumatically replanted into the sockets immediately and after 1-hour bench-drying, respectively.

Specimen Preparation

The jaw blocks containing the replanted roots and the surrounding periodontal tissues were harvested according to the representative healing phases at ½-, 2-, 3- and 4-day periods. The specimens were fixed, decalcified, processed for embedment in paraffin wax, serially cross-sectioned at 5-µm thickness, 100-µm intervals and prepared for immunohistochemistry according to the standard

protocols. The sections were stained with primary antibody, rabbit polyclonal antihuman IGF-I (ab9572, Abcam Ltd, Cambridge, UK) at the concentration of 1:50 polyclonal IGF-I antibody with 10% PBS at 4°C. The Xam-mounted slides were then subjected to immunohistomorphometric assay modified from the established histomorphometric evaluation method.³

Immuno-histomorphometric Assay

The specimens were evaluated using a light microscope (Nikon Optiphot II, Tokyo, Japan) at x40 and x400, with images projected onto a monitor (JVC, Yokohama, Japan) superimposed on a four 45° angle radii grid. With the center of the grid coinciding with the center of the root canal in the labiolingual axis, the 3 periodontal structures (cementum, PDL and bone) at the 8 intersection points along the root circumference were evaluated by 2 independent prior-calibrated examiners.

Statistical Analysis

The percentage of immunopositive cell counts and the mean extracellular intensity score, based on a pre-formulated visual analog scale (range, 0 to 3) (Fig. 1), were computed. Further analysis using Kruskal-Wallis and Mann-Whitney U tests with *P* value set at ≤0.05 were used to compare each periodontal structure over the 4 time points; the 3 periodontal structures at each time point within each experimental group; as well as the non-experimental group and the 2 treatment groups at each time point.

Results

The results are summarised in Figure 2. In bone, the lowest percentage cell count in the immediate replantation group at day 2 (89.05 ± 5.10%) was significantly lower than at day 4 and the non-experimental group (*P* = 0.00). There were also significant differences in percentage of cell counts between PDL and bone at day 2 (*P* ≤ 0.01).

IGF-I was least expressed in bone extracellular matrix in delayed replantation group at day ½ (1.29 ± 0.17) compared to day 3 (1.95 ± 0.43) and day 4 (1.72 ± 0.31) as well as the controls (1.66 ± 0.17) (*P* < 0.01). The extracellular bone intensity scores in the immediate replantation group at day ½ (1.57 ± 0.23) and day 3 (1.42 ± 0.37) were significantly different from that of delayed replantation group (*P* = 0.029; 0.008).

Discussion

The 100% IGF-I-positive cell counts and the mild to moderate immuno-stained extracellular matrix in all 3 periodontal structures suggested that IGF-I is stored in the cementum, PDL and bone under the physiologic condition.

It was observed that, cementum, being avascular mineralised connective tissue, appeared to play less significant role, compared to the vascularised bone and PDL, in the modulation of periodontal healing following tooth replantation.

The findings that the percentage of immunopositive-PDL cells and

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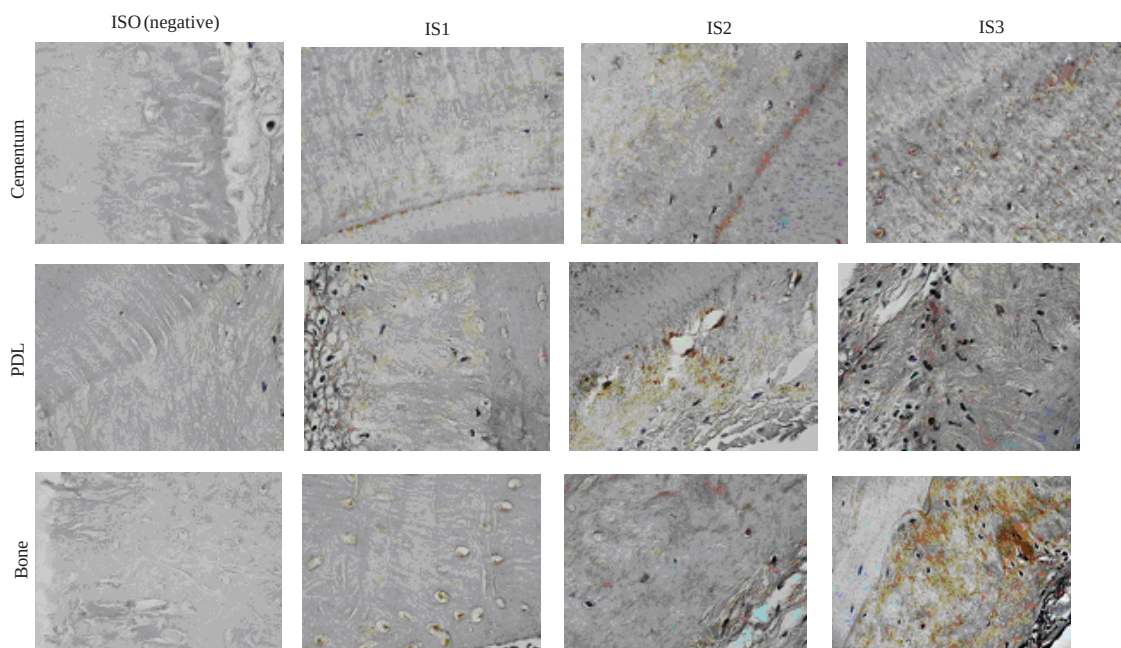


Fig. 1. Visual analog scale of intensity scores for IGF-I: Intensity Score (IS) 0 (negative), IS 1 (mild), IS 2 (moderate) and IS 3 (intense) for cementum, periodontal ligament and bone. Immunohistochemical staining with DAB chromogen, counterstained with haematoxylin.

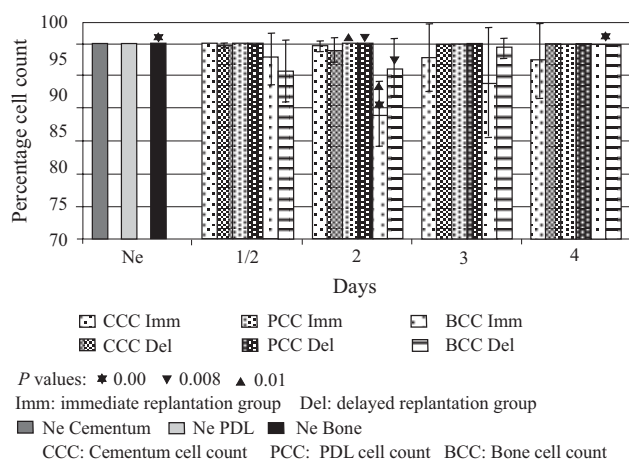


Fig. 2a. Comparison of percentage of cell counts in cementum, periodontal ligament and bone between immediate and delayed replantation groups at different observation times.

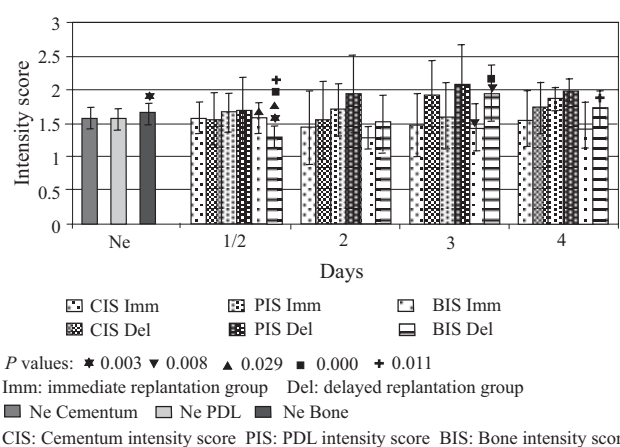


Fig. 2b. Comparison of intensity scores in cementum, periodontal ligament and bone between immediate and delayed replantation groups at different observation times.

the extracellular intensity in the 2 replantation groups at all observation timing were consistently near to the physiologic baseline, despite direct injuries incurred within PDL during the extraction/avulsion, substantiated the inherently high PDL turnover rate and the mitogenicity of IGF-I for PDL fibroblasts.²

With the ongoing bone remodelling, the decrease from the physiologic baseline in the percentage of IGF-I-positive-bone cells at day 2 and the subsequent normalisation at day 4 implicated the participation of paravascular progenitor cells within 48 hours.⁴ The significant decrease in extracellular intensity at day 1/2 compared to the physiologic baselines and to day 3 and day 4 delayed replantation group may be due to stimulation of osteoblastic proliferation, inducing noncollagenous proteins such as bone sialoprotein and osteopontin⁵ in the extracellular matrix.

In the delayed replantation group, the blood clot formed in the 1-hour socket could have served as a biological barrier to IGF-I rich plasma.⁶ This might explain the initial significantly low IGF-I expression in bone matrix at day 1/2 with the subsequent up-regulation at day 3, signalled by intensified inflammatory response perpetuated by the 1-hour desiccated necrotic PDL cells as the clot gradually replaced by granulation tissue. High IGF-expression correlated well with the area of injury where demand for cellular repair and regeneration is.

Conclusion

The early temporal and spatial IGF-I expression profile appears to be implicated in periodontal healing following tooth replantation.

Acknowledgement

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Laboratory Based) – Ethicon Surgical Book Prize (Laboratory)

Early Detection of Epithelial Ovarian Cancer Using a Proteomics-based Protein-Proteotyping Approach Combined with a Novel Selection Strategy

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Introduction

Ovarian cancer (OC) is the fifth most common cancer among women. The American Cancer Society estimates that about 25,580 new cases of OC will be diagnosed in the US during 2004. About 85% of OCs are epithelial ovarian carcinomas (EOC). The current survival of patients with EOC is low (5-year survival = 30%). The strategy that can help these patients is early diagnosis (Stage 1, 5-year survival = 90%).

The presence or abundance of specific molecules (biomarkers) in a biological fluid signals disease.^{1,2} The conventional approach relies on detection and monitoring of solitary markers in diagnostics and is not always reliable. The standard screening tool for EOC, the CA125, lacks sensitivity and specificity.³ Recently, newer classes of biomarkers derived from mass spectrometry analysis of low molecular weight proteome have shown improved results in early detection of EOC.⁴

We hypothesise that early EOC has a distinctive plasma/serum protein-profile signature (PPS) that allows it to be distinguished from normal individuals, and from patients with benign ovarian cysts (BOC) and late EOC. To support this, we have developed 2 diagnostic processes based on PPS that allow 100% accurate diagnosis of these 4 categories of patients without any misdiagnosis.

Material and Methods

A total of 129 plasma and 109 serum samples were analysed: early EOC (7,13), healthy controls (35,33), BOC (49,49), late EOC (18,34). The first number in brackets corresponds to serum samples, the second to plasma samples. Proteins from plasma and serum samples were analysed using surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF MS). Different chip surface chemistries (NP20, IMAC, SAX2, WCX2, H4) were tested and the hydrophobic H4 chip was selected as proteins detected on the H4 chip were the most numerous when compared to other chips in the selected molecular weight range of 0 to 20 kDa. The samples were processed according to the recommended

Ciphergen procedures.

After spectra have been obtained, they were processed and analysed as explained in the following section on Bioinformatics Analysis.

Bioinformatics Analysis

We developed a new method to correctly diagnose patients with the 4 categories based on serum and plasma protein spectra (PS). The method relied on combinations of proteomic patterns (PPs) determined from PS. Each spectrum contained a total of 22,500 data points. A baseline correction was made to reduce the noise in spectra. Essentially, the feature selection was based on selecting those PPs that were the least mutually correlated and whose combination produced the best separation between the considered classes based on linear discriminant models. For training, a very small fraction of available data was used (Table 1) while the remaining data was used for blind testing. We attempted to use as few PPs as possible while ensuring accurate diagnosis.

Results

We established hierarchical diagnosis strategies, as shown in Fig. 1, which resulted in 100% accuracy (sensitivity = 100%, specificity = 100%) in diagnosis of cases from both the training data and the test data used as blind tests. Diagnostic processes are illustrated in Figure 1. Three diagnosis steps were required for serum or plasma PS to produce correct diagnosis of all cases (early EOC, late EOC, benign, normal). The diagnostic processes are illustrated in Figure 1 and Table 1. In plasma based PS, cancer cases were first separated from non-cancer cases. In the cancer cases, early-stage EOC cases were separated from the late-stage EOC. Similarly for non-cancer cases, benign cases were separated from normal, thus allowing complete diagnosis of patients into the 4 categories. The process was applied to serum based PS as per Figure 1. PPs from serum and plasma constituents of blood showed distinct appearance of proteins for each of the 4 categories. Majority of the proteins were identified in the region between 7 and 12 kDa. These were used in the

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Table 1. Diagnosis Results Produced on the Training and Test Data for Serum Based Proteomic Spectra and for Plasma Based Proteomic Spectra

Serum based proteomic spectra						
Non-cancer		Cancer			Model type	Features
Normal	Benign	Early	Late	Unclear		
35	49	7	18	7		
4		8			Training set 1 (cancer + benign vs. normal)	9
31		68			Test 1 (cancers + benign vs. normal)	9
	10		3		Training set 2 (cancer vs. benign)	12
	39		22		Test 2 (cancer vs. benign)	12
		2	2		Training set 3 (early vs. late)	2
		5	16		Test 3 (early vs. late)	2
Plasma based proteomic spectra						
Non-cancer		Cancer			Model type	Features
Normal	Benign	Early	Late	Unclear		
33	49	13	34	7		
	10		5		Training set 1 (cancer vs. non-cancer)	8
	72		42		Test 1 (cancer vs. non-cancer)	8
		2	8		Training set 2 (early vs. late)	8
		11	26		Test 2 (early vs. late)	8
3	5				Training set 3 (benign vs. normal)	12
30	44				Test 3 (benign vs. normal)	12

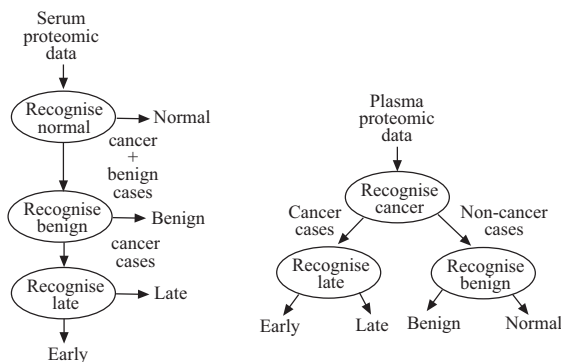


Fig. 1 Illustration of diagnostic processes in cases when diagnosis is made based on serum or plasma spectra. Different diagnostic procedures are applied for data from serum and from plasma. Diagnostic models in both cases are applied 3 times in order to diagnose all possible cases. However, diagnostic models are developed for different groups of patients and used different proteomic patterns in the diagnosis decision. The final goal is to diagnose patients with the correct category out of 4 (early EOC, late EOC, benign, normal). The nodes in the diagnosis processes depicted represent points where decisions are made. The text next to branches indicates which cases have been distinguished by the diagnostic process.

generation of diagnostic models (DMs) which are illustrated in the case of plasma data (Table 1). To separate cancer from non-cancer cases, 10 non-cancer and 5 cancer cases were randomly selected, and DM trained to separate them. The number of selected PPs was increased from 1 to 8, until perfect diagnosis was achieved. Two cases from early EOC and 8 cases from late EOC were used to train DM to separate these cases and required 8 PPs. Analogously, we used 3 normal and 5 benign cases to train DM to separate benign from normal cases with 12 PPs. The resultant DMs and the diagnostic process produced perfect diagnostic results on both the training and test data without any misdiagnosis.

Discussion

Our goal is not only to apply these biomarkers for the early

diagnosis of EOC (benign or malignant), but also to distinguish benign from malignant disease. The novelty of this approach is the minimum number of features required to discriminate different stages of cancer.

Previous reports were not based on the clinically tested data as done in our study based on several supporting evidences.⁵ Our approach is more of diagnosis type and not of screening as was focus of previous studies.

We have used both serum and plasma proteins for the first time to discriminate different classes and stages of EOC. However, we were not able to identify a biomarker that correlated in size to CA125. We speculate that CA125 may not bind to the H4 or other chips used in our studies or that its large size is not conducive to ionisation and flight. Majority of the markers identified were found in the low molecular weight range in the region between 7.5 kDa and 10 kDa, similar to previous reports.⁶

In conclusion, we have discovered a novel selection strategy based on protein profiling technology that could significantly improve the early detection of EOC.

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Non-invasive Prenatal Diagnosis of Fetal Gender Using Real-time Polymerase Chain Reaction Amplification of *SRY* in Maternal Plasma

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Introduction

In Singapore, 1 in 5 pregnancies occur in mothers >35 years old and genetic diseases are common. Without prenatal diagnosis, 1 in 50 babies are born with serious physical or mental handicap, and as many as 1 in 30 with some form of congenital malformation. Current methods for the diagnosis of aneuploidy and monogenic disorders require invasive testing by amniocentesis, chorion villus biopsy or fetal blood sampling. These tests carry a procedure-related risk of miscarriage of 1% to 4% that is unacceptable to many couples. Non-invasive methods of prenatal diagnosis are desired to both patients and clinicians. A novel approach for non-invasive prenatal diagnosis is the use of cell-free fetal DNA in the serum or plasma of pregnant women. The presence of fetal DNA in maternal plasma was first demonstrated by Lo et al in 1997.¹ He showed that fetal DNA constitutes a mean 3.4% (range, 0.39% to 11.9%) of the total plasma DNA in the first trimester and a mean of 6.2% (range, 2.33% to 11.4%) in the third trimester.² This would mean that fetal genetic material can be obtained in first trimester maternal plasma for genetic analysis. We successfully isolated cell-free fetal DNA from maternal plasma in as early as 8 gestational weeks. Using real-time polymerase chain reaction (PCR), we were able to amplify and quantify the isolated fetal DNA. Fetal gender is also determined with 100% accuracy using this novel non-invasive prenatal diagnosis technique.

Materials and Methods

Sample Collection and DNA Isolation

Sample collection for research was approved by the Institutional Review Board. Two millilitres of maternal peripheral blood were collected in EDTA tubes after written informed consent from 23 mothers undergoing amniocentesis, ultrasound or termination of pregnancy (TOP) between 6⁺⁵ to 36⁺⁴ gestational weeks. Trophoblast tissues were collected from TOP for molecular genetic analysis of fetal gender. All blood samples were processed within 2 hours of sampling. The peripheral blood was centrifuged at 2760 rpm for 10 minutes and at 13,000 rpm for another 10 minutes to obtain the plasma fraction for DNA isolation. DNA was isolated from 800 µL of plasma using the High Pure PCR Template Preparation Kit according to the manufacturer's recommendations (Roche Diagnostics GmbH, Germany).

Quantitative Measurement and Fetal Gender Determination from DNA Isolated from Maternal Plasma using Real-time Quantitative PCR

Real-time PCR analysis was performed by the use of a PE Applied Biosystems 7000 Sequence Detector. *β-globin*, a chromosome 11 locus, was used as the endogenous control for the quantitative measurement of total DNA. The Y chromosome *SRY* (testis-determining factor) locus was used for the quantitation of fetal DNA and fetal gender determination. The sequence of primers and probe combinations were described by Lo et al.² Positive controls for male gender determination were commercial male genomic DNA while

commercial female genomic DNA were used as the negative controls. The commercial male genomic DNA was serially diluted 5-fold and set as standards to generate the standard curve for absolute quantitation. Each sample and standard was run in triplicates with both sample and standards running in parallel.

Fluorescence in situ Hybridisation (FISH) of Trophoblast Tissues for Fetal Gender

In all cases of TOP, FISH of trophoblast cells was used to confirm fetal gender. We used centromeric enumeration CEP® X/Y probe (Vysis Inc, Downers Grove, Illinois, USA) to label the sex chromosomes for fetal gender analysis.

Results

Qualitative Analysis

Of the 23 maternal plasma samples analysed, 13 male and 10 female fetuses were identified by real-time PCR amplifications of *SRY* and *β-globin*. Endogenous controls (*β-globin*) amplified in all reactions (100%) and *SRY* was amplified in 13 maternal plasma samples. For samples obtained from mothers undergoing amniocentesis, fetal genders were confirmed by cytogenetic karyotypes. Ultrasound results were confirmed at birth. FISH of trophoblast cells obtained from TOP confirmed fetal gender. In all cases (100%), fetal gender was concordant between *SRY* amplification and known fetal gender (chi-square 23.0; Fisher's exact *P* < 0.001).

Quantitative Analysis

The conversion factor of 6.6 pg of DNA per cell was used, for expression of results as genome equivalents (GE). Total DNA concentrations in maternal plasma (measured by *β-globin* amplifications) ranged from 355.4 to 4642.4 GE/mL (median = 1653.0 GE/mL). Fetal DNA concentrations determined from *SRY* amplifications ranged from 3.04 to 169.00 GE/mL (median = 10.97 GE/mL).

Sensitivities of *SRY* and *β-globin* Assays

The lowest serial dilution of commercial male genomic DNA is 1.5 GE/mL, which is amplified and detected by both *SRY* and *β-globin* real-time PCR assays in all runs.

Standard Curves

Regression coefficients for all runs were at -0.99 (ideal -1.00). For *SRY* standard curves, the mean ± SD slope was -3.55 ± 0.16 with the mean ± SD intercept of 38.11 ± 0.49. For *β-globin* standard curves, the mean ± SD slope was -3.52 ± 0.13 with the mean ± SD intercept of 37.13 ± 0.78.

Discussion

In this study, we had shown: (1) 100% accuracy in fetal gender determination using maternal plasma in 3 trimesters (Table 1); (2) that cell-free fetal DNA in maternal plasma can be quantified in as early as 8⁺² gestational weeks (Table 1); (3) that fetal DNA concentration in maternal plasma increased as pregnancy progressed

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Table 1. Determination of Fetal Gender from Maternal Plasma

Sample	Gestational weeks	Real-time PCR results	Confirmatory results
1	6+5	Female	XX
2	7+6	Female	XX
3	8+2	Male	XY
4	8+5	Female	XX
5	8+5	Male	XY
6	9+0	Male	XY
7	9+3	Female	XX
8	16+4	Male	XY
9	21+5	Female	XX
10	21+6	Female	XX
11	22+0	Male	XY
12	24+0	Male	XY
13	32+0	Female	XX
14	32+0	Male	XY
15	32+0	Male	XY
16	33+1	Male	XY
17	34+1	Male	XY
18	35+0	Male	XY
19	35+4	Female	XX
20	36+0	Female	XX
21	36+0	Male	XY
22	36+2	Male	XY
23	36+4	Female	XX

Out of 23 maternal plasma samples (gestational weeks ranged from 6⁺⁵ to 36⁺⁴), 13 are determined as male while 10 are determined as female fetuses. Real-time PCR results are 100% concordant with confirmatory results obtained from conventional karyotype, fluorescence in situ hybridisation and at birth.

(from a median of 11.1 GE/mL in the first trimester to a median of 84.8 GE/mL in the third trimester) (Table 2); (4) with a detection limit of 1.5 GE/mL, the sensitivities of our *SRY* and *β-globin* real-time PCR assays are high. The lowest fetal DNA concentration that we had quantified from maternal plasma was 3.04 GE/mL (Table 2).

Cell-free fetal DNA in maternal plasma provides an alternative source of fetal genetic material without the need for invasive procedures. As demonstrated in this study, this source of fetal DNA can be used for fetal gender determination. Other genetic analysis that is feasible with this non-invasive prenatal diagnosis technique includes assessment of sex-linked disorders, the detection of unique

Table 2. Relationship of Gestational Age with Fetal DNA and Total DNA Concentrations in Maternal Plasma

	SRY concentration (GE/mL)		
	1 st trimester	2 nd trimester	3 rd trimester
Range	11.0-26.2	3.0-129.2	67.4-121.6
Mean	16.1	56.8	100.1
Median	11.1	38.0	84.8
	β-globin concentration (GE/mL)		
	1 st trimester	2 nd trimester	3 rd trimester
Range	355.4-2544.1	722.4-2443.4	802.8-4642.4
Mean	1413.73	1424.3	2352.3
Median	1476.67	1171.2	2315.2

GE/mL: genome equivalents per millilitres of maternal plasma

gene sequences such as RhD locus to determine fetomaternal blood group incompatibility³, and the detection of dominantly inherited, paternally derived mutations for single gene disorders diagnosis.^{4,5} Advances in technology had also allowed researchers to expand the genetic analysis of fetal DNA in maternal plasma to include more mutations.⁶

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Neuropsychological Correlates of Hippocampal Volumes in First Episode Psychosis

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Introduction

There is increasing evidence that schizophrenia is a brain disorder and numerous neuroimaging studies show brain abnormalities in schizophrenia patients. Magnetic resonance imaging (MRI) studies have linked hippocampal abnormalities in the pathophysiology of psychosis (Bogerts et al, 1990; Bogerts et al, 1993). A meta-analysis of 18 structural MRI studies (Nelson et al, 1998)¹ reported a 4% reduction in bilateral hippocampal volumes in schizophrenia patients compared to normal controls.

As highlighted by several reviews (Moser et al, 1999; Strange et al, 1999), there are neuroanatomical and functional differences between anterior and posterior hippocampus projections. While the anterior hippocampus is predominantly connected to other limbic and striatal systems, the posterior hippocampus receives converging sensory input from posterior cortices. In fact, Suddath et al (1990) and Weinburger et al (1992) found abnormalities in anterior hippocampus in relation to other parts of the hippocampus between monozygotic twins discordant for schizophrenia.

However, as most of these studies involved chronic patients, their findings could be confounded by chronicity of the illness and long-term medication effects. Investigating patients with first-episode psychosis circumvents the problem and allows us to look at structural brain changes directly relevant to the disease process.

In attempting to understand how neuroanatomical abnormalities translate into cognitive performance, studies have investigated the neuropsychological profile of patients with first-episode psychosis (Censits et al, 1997; Bilder et al, 2000; Addington et al, 2003).² They found that patients showed deficits in domains such as verbal fluency, executive function, attention and memory.

Few have looked at neuropsychological performance and anterior versus posterior hippocampal volumes in first-episode patients. Studies by Bilder et al (1995)³ and Szeszko et al (2002)⁴ found an association between smaller anterior hippocampus and worse executive and motor functions. This study aims to look at neuropsychological correlates of hippocampal volumes in Asian patients with first-episode psychosis.

Materials and Methods

Consecutive patients accepted into the Early Psychosis Intervention Programme (EPIP) were approached for participation. Exclusion criteria were neurological problems, significant medical illness, alcohol or drug abuse and mental retardation. Diagnosis was made using the Structured Clinical Interview for DSM-IV diagnoses (SCID). Duration of psychosis (DUP) was determined from time of onset of first psychotic symptoms to time of definitive diagnoses as assessed from structured interviews with patients and relatives. Written informed consent was obtained and patients were scanned within 4 weeks of starting antipsychotics. Patient demographic and clinical details are provided in Table 1.

Table 1. Patients' Demographic and Clinical Characteristics

Variable	No. of patients
Gender	
Female	5 (35.7%)
Male	9 (64.3%)
Race	
Chinese	12 (85.7%)
Indian	2 (14.3%)
Diagnosis	
Schizophrenia	10 (71.4%)
Schizoaffective disorder	1 (7.1%)
Schizophreniform disorder	1 (7.1%)
Bipolar disorder with psychotic features	1 (7.1%)
Brief psychotic disorder	1 (7.1%)
	Mean (SD)
Age (y)	26.5 (6.6)
Duration of untreated psychosis (mo)	11.5 (11.6)
Years of schooling	11.6 (2.7)

MRI Data Acquisition and Image Analysis

Patients underwent imaging on a 1.5T MRI scanner (NVi, GE Medical Systems, Wisconsin, MI) with high resolution, fast gradient recalled (FGRE) 3D volumetric scans (TR/TE/TI/flip angle 6.4/1.5/400/20; matrix 256 x 256, FOV mm²) with coronal orientation, covering the whole brain for structural-anatomic detail.

Hippocampal volume measurements were performed using MRreg v.1.6.2 software by an experienced reader blinded to left-right anatomic status. Anterior-posterior hippocampal volume delineation was done using published procedure (Sullivan et al, 1995).⁵

Neurocognitive Battery

A neurocognitive battery was administered within 2 months when patients were deemed clinically stable by their clinicians. Tests used in the neurocognitive battery are shown in Table 2.

Results

Fourteen patients were recruited. Spearman's partial correlations examined the relationship between hippocampal volume and

Table 2. Tests Used in Neuropsychological Battery

Premorbid Cognitive Functioning
National Adult Reading Test (NART)
Current Cognitive Functioning
Raven's Progressive Matrices (RPM)
Attention
Continuous Performance Test (CPT)
Digit Span, Wechsler Adult Intelligence Scale – 3 rd edition (WAIS – III)
Executive Functioning
Trail-making Test, Parts A and B
Wisconsin Card Sort Task (WCST)

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neuropsychological tests, controlling for age, gender and years of education.

Significant correlations were found for scores on Trails Part A and left anterior hippocampal volume ($r = 0.81$, $P < 0.05$), and Trails Part B and total anterior hippocampal volume ($r = 0.76$, $P < 0.05$), suggesting better performance with bigger hippocampus. No other significant correlations were found.

Discussion

Even upon first presentation, our first-episode patients had significant impairment on tasks of executive function. In particular, anterior hippocampal volume was significantly correlated with executive functioning, which is not explained by years of education. This is consistent with results obtained by Bilder et al (1995)³ and Szeszko et al (2002).⁴ The association of executive functioning deficits with reduced anterior hippocampus is also compatible with structural and functional-anatomic data relevant to schizophrenia.

Our study sampled first-episode psychosis patients with no long-term medication or a history of substance abuse. Further research

using in vivo functional Magnetic Resonance Imaging (fMRI) techniques will shed more light on this structural-functional link.

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Special Poster Presentation

Nuclear Factor kappa B Transcription Profiling of Genes Protect Against Nitric Oxide-induced Neuronal Apoptosis

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Introduction

Nitric oxide (NO) is a multifaceted molecule that is involved in a wide range of physiological and pathophysiological processes. It is also implicated in a variety of neurological disorders like stroke, seizure and Alzheimer's disease.

Nuclear Factor kappa B (NF- κ B) is a transcription factor that contributes to synaptic transmission, neural development and is also involved in neurodegenerative diseases in the nervous system. In a previous report, we showed that NF- κ B plays a protective role against nitric oxide-induced apoptosis in neuroblastoma cell line, SHEP-1.¹ However, the neuroprotective mechanism of NF- κ B remains to be elucidated.

Neuronal cell death can also be modulated by other factors. Insulin factor-like growth factor (IGF-1) is a potent neurotrophic and anti-apoptotic factor which activates NF- κ B. In this study, the role of IGF-1 in NF- κ B-mediated neuroprotection of NO-induced apoptosis is investigated.

Material and Methods

Cell Culture

Human SHEP-1 neuroblastoma cells were maintained in Dulbecco's modified Eagle's medium containing 10% foetal calf serum, 100 units/mL penicillin, and 100 μ g/mL streptomycin at 37°C in humidified 5% CO₂. To clarify the role of NF- κ B in NO-induced apoptosis in SHEP-1, a stable transformed SHEP-1 neuroblastoma cells expressing I- κ B α M, deviating from I- κ B α that

binds and inhibit NF- κ B was created using LipofectAMINE and maintained with 500 μ g/mL G418.

NF- κ B p65 Activator Assay

SHEP-1 cells were treated with IGF-1 at the indicated time frames. Proteins were harvested and subjected to the reporter assay according to the protocol of TransAM NF- κ B p65 activator assay (Active Motif). Protein concentration was determined by Bio-Rad protein assay.

Determination of Cell Viability

Neuronal cell viability was assessed by crystal violet staining where SHEP-1 and I- κ B α M cells were grown in 96-well plates in triplicates and treated with SNP for up to 24 h.

cDNA Microarray

RNA of the I- κ B α M and IGF-1 treated SHEP-1 were harvested and subjected to a human 12K cDNA microarray (BD Biosciences Clontech).

RT-PCR and PCR

Total RNA was harvested from IGF-1 treated cells using Trizol reagent. The reverse transcription and polymerase chain reaction amplification (RT-PCR) from total RNA was performed as described in SuperscriptTM III first strand synthesis system for RT-PCR. PCR amplification was performed using the primers of biglycan, Matrix Gla protein and Tenascin C with actin as control.

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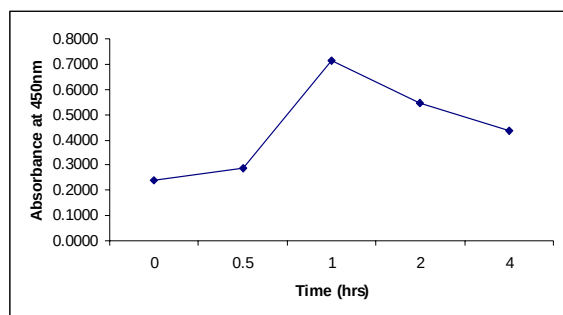


Fig. 1a.

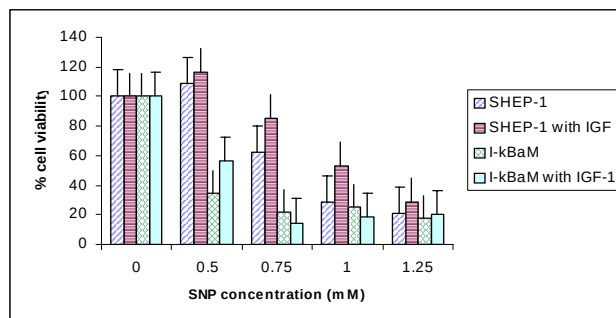


Fig. 1b.

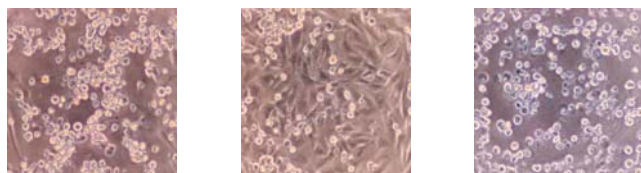


Fig. 1c.

Fig. 1. IGF-1 activates NF- κ B and protects SHEP-1 cells against NO induced apoptosis. (a) IGF-1 treated SHEP-1 nuclear protein is subjected to NF- κ B p65 activator assay where the absorbance is measured at wavelength of 405 nm with reference wavelength of 650 nm. (b) SHEP-1 and I-kBaM cells were exposed to the indicated SNP concentrations in the presence or absence of IGF-1 (200 ng/mL) for 24 hours. Cell viability was quantitated using crystal violet staining. (c) Photos of SHEP-1 cells treated with 0.75 mM NO (left), 0.75 mM NO and 200 ng/mL IGF-1 (middle) and I-kBaM cells treated with 0.75 mM NO and 200 ng/mL IGF-1 (magnification x400).

Increased gene expression

Anti-apoptosis

Biglycan
Glutathione peroxidase 3 (plasma)

Growth/Repair/Differentiation

Carbonic anhydrase IX
Serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1
Colony stimulating factor 1 (macrophage)

Immune/Inflammation

Complement component 1, r subcomponent
Leukotriene b4 receptor (chemokine receptor-like 1)
Hypothetical protein FLJ10143

Signal transduction/Transcription

G protein-coupled receptor 38
RNA-binding protein gene with multiple splicing
G protein-coupled receptor 27

Metabolism

Transmembrane 7 superfamily member 2
Stearoyl-CoA desaturase (delta-9-desaturase)
Elastin microfibril interface located protein

Structural

EGF-containing fibulin-like extracellular matrix protein 1
Actinin, alpha 1
Actin related protein 2/3 complex, subunit 1A (41 kD)

Channels/Transporters

Solute carrier family 22 (organic cation transporter), member 4
Selenium binding protein 1
Potassium voltage-gated channel, Isk-related family, member 1

Hormone/Neurotransmitters

Adenylate cyclase activating polypeptide 1 (pituitary)
Carboxypeptidase E
Prodynorphin

Electron transport

Oxidase (cytochrome c) assembly 1-like
Protease inhibitor 3, skin-derived (SKALP)
Hypothetical protein LOC51061

Other proteins

Matrix Gla protein
Glutamate-ammonia ligase (glutamine synthase)
Hypothetical protein PRO2121

Decreased gene expression

Signal transduction/Transcription
Arg/Abl-interacting protein ArgBP2

Metabolism

Adenosine deaminase

Hormones/Neurotransmitter

Adrenomedullin
Channels/Transporters
Retinol binding protein 1, cellular
Glutamate receptor, ionotropic, kainate 3
Potassium inwardly-rectifying channel, subfamily J, inhibitor 1

Structural

Bicaudal D homolog 1 (Drosophila)
Other proteins
Nucleoredoxin 1
Protein expressed in thyroid
ATP synthase, H⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2
Palmitoyl-protein thioesterase 2
U6 snRNA-associated Sm-like protein LSm7
Guanine nucleotide binding protein 11
Hypothetical protein HSN44A4A
CGI-63 protein
ATP synthase, H⁺ transporting, mitochondrial F0 complex, subunit f, isoform 2
ATPase, H⁺ transporting, lysosomal 14kD, V1 subunit F
Stem cell growth factor; lymphocyte secreted C-type lectin
CGI-67 protein
Serine/threonine kinase 12
Tumor necrosis factor, alpha-induced protein 2

Fig. 2a.

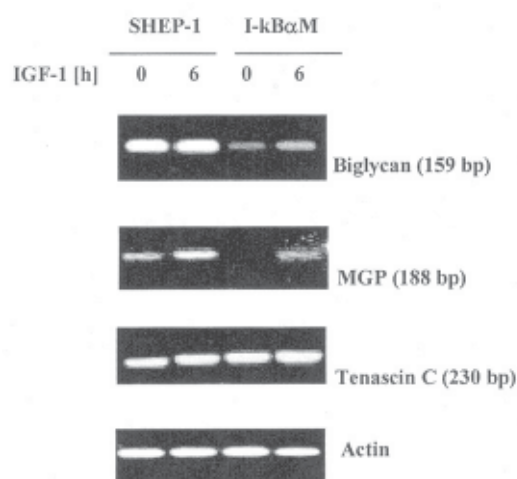


Fig. 2b.

Determination of Biglycan in Cell Culture

SHEP-1 and I-kBαM cells were treated with 200 ng/mL IGF-1 in serum-free DMEM medium. The medium was then collected, filtered and digested using 100 mU of chondroitinase ABCase for 16 h. Proteins were concentrated by incubating the medium with StrataClean™ resin, resuspended in 2 x SDS sample buffer and subjected to Western Blot analysis.

Western Blot Analysis

Biglycan protein was resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane and probed with primary antibody against biglycan in PBS-T (provided by Dr KikuchiA, NCNP, Japan). After washing with PBS-T, the membrane was probed with horseradish peroxidase-conjugated antiserum to rabbit and developed by the enhanced chemiluminescence's method.

Results

IGF-1 activates NF-κB in SHEP-1 cells

We have previously shown that IGF-1 induces the activation of NF-κB in SHEP-1 cell but not in I-kBαM cells using immunocytochemical staining of p65 subunit.¹ NF-κB reporter assay performed showed that NF-κB activity started increasing at 30 min, reached its peak at 1 h and decreased substantially after 2 h of IGF-1 treatments confirming NF-κB activation in SHEP-1 cells by IGF-1 (Fig. 1a).

Neuroprotective effects of IGF-1 against NO-induced apoptosis in an NF-κB-dependent manner

The effects of IGF-1 in NO-induced apoptosis were later examined. As seen in Figure 1b, I-kBαM cells were more sensitive to apoptosis induced by varying SNP concentrations as compared to control SHEP-1 cells. The administration of 200 ng/mL IGF-1 promoted the survival of NO-treated SHEP-1 in a dose dependent manner but not the case with I-kBαM cells (Fig. 1b and Fig. 1c). These results suggest that IGF-1 protects SHEP-1 cells against NO-induced apoptosis in an NF-κB dependent manner although the actual mechanism is still unclear.

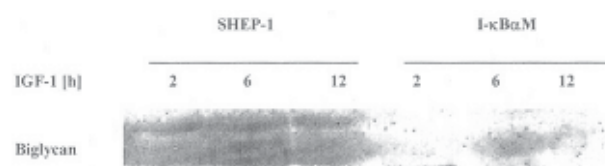


Fig. 2c.

Fig. 2. Analysis of cDNA microarray results. (a) RNA was extracted from 200ng/mL IGF-1 treated SHEP-1 cells treated with 200 ng/mL IGF-1 and I-kBαM cells and subjected to 12K human cDNA microarray analysis. Altered expression of genes that had more than 5 fold are divided into different categories. (b) The gene expression of biglycan, Matrix Gla protein and Tenascin C were evaluated using RT-PCR with actin as internal control. (c) Immunoblotting analysis of biglycan from IGF-1 treated SHEP-1 and I-kBαM cell culture medium at the indicated time frames.

cDNA Microarray Results

To elucidate the genes that are involved in the protective role of IGF-1 in NO-induced apoptosis in SHEP-1, cDNA of I-kBαM cells and IGF-1 treated SHEP-1 were harvested for cDNA microarray analysis. The results were analysed and only genes with more than 5-fold change in expression were listed and categorised in Figure 2a. As seen in Figure 2b, the administration of IGF-1 upregulated biglycan, Matrix Gla protein mRNA in SHEP-1 cells as compared to those in I-kBαM cells.

IGF-1 Upregulates Biglycan in SHEP-1 Cells

Biglycan was shown to be nitric oxide-regulated and to promote the survival of mesangial cells.² We investigated the protein levels of biglycan in IGF-1 treated SHEP-1 and I-kBαM cells using immunoblot analysis. Biglycan was upregulated for the first few hours and reached its peak at 6 h post treatment of IGF-1. However, with prolonged exposure of up to 12 hours, Biglycan was shown to decrease substantially (Fig. 2c). Similar trend of results were observed in IGF-1 treated I-kBαM cells although the levels of biglycan was much reduced. These results suggest that biglycan might be one of the genes targeted by NF-κB in triggering off the NF-κB-mediated neuroprotection mechanism.

Conclusion

In this study, we have shown that IGF-1 is involved in the neuroprotection against NO-induced apoptosis in a NF-κB-dependent manner with altered expression of various genes like Matrix Gla protein and biglycan. This information obtained can potentially be used in therapeutic approaches in neurological disorders.

Acknowledgement

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Comparison of Body Mass Index and Subjective Global Assessment as Indices of Nutrition in Hospital Inpatients

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Introduction

There are many different indices of nutrition available for assessing nutritional status and diagnosing malnutrition. Some are easily performed, readily available and inexpensive, whereas, others are sophisticated, not available and expensive.¹ Anthropometric methods are commonly used to describe nutritional status as it provides an overview of the human body composition.² Weight, height and the derived body mass index (BMI) are easily available, inexpensive and practical indices. In adults, weight is usually stable and a change in weight suggests an abnormal process, nutritionally or otherwise.¹ Another practical method is Subjective Global Assessment (SGA). This is a clinical method for evaluating nutritional status that includes a patient's weight history, symptoms as well as physical parameters.² In this study we compared the use of the BMI and Subjective Global Assessment in patients admitted to an acute medical ward to evaluate their sensitivity in diagnosing malnutrition.

Materials and Methods

This was a prospective study conducted from 1 to 31 August 2003 in an acute admitting renal ward in a tertiary hospital in Singapore. In total, 55 patients who had both their BMI and Subjective Global Assessment evaluated by 2 dietitians were included in the study. Patients with oedema were excluded, as their dry weight was difficult to ascertain.

The mean age ($\pm$ SD) of the subjects was 57.4 ± 14.5 years. Sixty-six per cent were diabetic and 58% had end-stage renal failure. Twenty-nine percent were males. Forty-five per cent were Chinese, 31% (Malay), 20% (Indian) and 4% (Others).

Body Mass Index: This was calculated using the formula: weight (kg)/height² (m). A BMI of <18.5 was considered underweight, 18.5 to 24.9 as normal weight and ≥ 25 as overweight. A BMI of <18.5 was considered indicative of malnutrition.

Subjective Global Assessment (SGA): A revised 7-point scale Subjective Global Assessment was used.⁴ Subjective Global Assessment scores ranged from 1 to 7 with a score of 1 indicating severe malnutrition and 7 indicating normal nutrition. A Subjective Global Assessment of 6 or less was considered indicative of malnutrition.

All data were analysed using SPSS version 11.5. BMI and Subjective Global Assessment scores were cross-tabulated and the significance of the distribution was measured using the Chi-square test. *P* value of <0.05 was taken as statistically significant.

Results

The comparison of BMI and Subjective Global Assessment score data are shown in Table 1. Mean height ($\pm$ SD) of the patients was $1.57 (\pm 0.08)$ metres and mean weight ($\pm$ SD) was $60.62 (\pm 13.87)$ kg.

Three patients out of 55 (5%) had BMI <18.5 . Of these, 2 out of 3 patients (67%) had Subjective Global Assessment <6 . Twenty-six patients out of 55 (47%) had a Subjective Global Assessment <6 . Of these, only 2 out of 26 patients (7.7%) had a BMI of <18.5 . Only 2

Table 1. Comparison of BMI^a and SGA^b Score in 55 Patients

SGA score	BMI <6	BMI ≥ 6	Total
<18.5	2	1	3
18.5 – 24.9	17	11	28
≥ 25	7	17	24
Total	26	29	55

The value for *P* ≤ 0.05 , Pearson's R.

out of 55 patients (4%) had a BMI <18.5 and a Subjective Global Assessment of <6 . This distribution is statistically significant (*P* < 0.05).

Discussion

This study shows that Subjective Global Assessment usage may detect a higher incidence of malnutrition compared to the use of BMI.

BMI seems to be less sensitive in detecting malnutrition. This may be because it does not take into account a patient's weight and dietary history, symptoms like nausea, vomiting or diarrhoea, functional status relating to nutrition and physical parameters. In addition, although metabolic changes in relation to energy and protein deficiency can occur within hours or days of reducing nutrient intakes, anthropometric changes may take much longer.^{3,4} In an acute admitting hospital this may be very prevalent. The changes in weight may not manifest. Therefore in this situation the Subjective Global Assessment may be a more sensitive indicator of malnutrition.

Nutritional status of patients is an important and modifiable factor that may influence disease processes and outcome.² Early detection and intervention is therefore important. An appropriate and sensitive method of assessment of malnutrition is therefore essential. This study shows that a method such as the Subjective Global Assessment which takes into account not only anthropometric measures like weight but also includes patient's symptoms and signs may be more appropriate.

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The Impact of Time-to-Balloon on Outcomes in Patients Undergoing Modern Primary Angioplasty for Acute Myocardial Infarction

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Introduction

Rapid time to treatment with thrombolytic therapy is associated with lower mortality in patients with acute ST-segment elevation myocardial infarction (STEMI).¹ However, the importance of time-to-primary percutaneous coronary intervention (PCI) in acute myocardial infarction (AMI) remains controversial. Cannon et al reported a delay in door-to-balloon time to be the major outcome predictor but not symptom-to-balloon time.² Other studies found symptom-to-balloon time to be more crucial.³ Going by “open artery” theory, the more rapidly reperfusion is achieved with primary angioplasty, the better the outcome and conversely, delays in achieving reperfusion result in higher mortality.

Our study aimed to evaluate, in a single-centre cohort of patients with STEMI, the relationship between delay in symptom-to-treatment and door-to-treatment time on short- to medium-term clinical outcomes

Materials and Methods

Our study is a single-centre, prospective observational study conducted at NUH, Singapore. Acute STEMI was defined as a patient with a history suggestive of AMI accompanied by electrographic (ECG) evidence of ST-segment elevation of at least 0.1 mV in 2 or more ECG leads. There were no exclusion criteria. Between June 2001 and May 2003, a total of 208 consecutive, unselected patients with STEMI and had underwent primary PCI (without antecedent fibrinolytic therapy) were included.

Symptom-to-balloon time was defined as the interval between the time of patient's reported symptom(s) onset and time of first balloon inflation. Door-to-balloon time was the interval between the time of patient registration at emergency department and time of first balloon inflation.

Our hospital has fully computerised patient database systems that include EMDS (Emergency Database System), CPSS (Computerised Patient Support System) and catheterisation laboratory database (4D client, © 4D, Inc. 1995-2004). All data were acquired from the above databases.

Patients were divided into several pre-specified groups, first by time from symptom onset to first balloon inflation, and then by time of door to first balloon time. Baseline characteristics, mortality rate and major adverse cardiac event (MACE) rates were examined across these time categories. The primary endpoints of this study were mortality rate and MACE rate at 1 month and 6 months post-event. MACE was defined as death, myocardial infarction and repeat target vessel revascularisation.

All statistical analysis was performed using SPSS 11.5. Univariate analyses were conducted to identify the variables linked to mortality and MACE across the different time categories. Multivariate analysis was performed using logistic regression with adjusting for appropriate covariates. Statistical significance was assumed if $P < 0.05$.

Results

The demographic and clinical characteristics of 208 patients

Table 1. Baseline Variables by Symptom-to-Balloon Time

Baseline variable	Symptom-to-balloon time				P Value
	<2 h (n = 7)	2-4 h (n = 100)	4-6 h (n = 48)	>6 h (n = 39)	
% of total patients	3.6%	51.6%	24.7%	20.1%	N/A
Age <70 years	7 (100%)	87 (87%)	39 (81.3%)	34 (87.2%)	0.537
Age (y) (mean ± SD)	50.1 ± 8.3	54.3 ± 12.3	59.3 ± 11.5	57.5 ± 10.6	0.042
Men	7 (100%)	92 (92%)	41 (85.4%)	33 (84.6%)	0.391
Diabetes mellitus	0 (0%)	19 (19%)	13 (27.1%)	13 (33.3%)	0.124
Hypertension	3 (42.9%)	49 (49%)	28 (58.3%)	24 (61.5%)	0.454
Current smoker	3 (42.9%)	51 (51%)	23 (47.9%)	20 (51.3%)	0.927
Prior CABG	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1.000
Prior MI	2 (28.6%)	7 (7%)	3 (6.3%)	5 (12.8%)	0.159
Anterior Wall Infarction	4 (57.1%)	60 (60%)	28 (58.3%)	19 (48.7%)	0.407

CABG: coronary artery bypass grafting; MI: myocardial infarct

Note: 14 (6.7%) missing data

according to time-to-balloon are shown in Table 1. The ethnic composition of patients consisted of Chinese (65%), Indian (18%), Malay (15%) and others (2%). Patients were predominantly male and aged less than 70 years old. Patients with a longer ischaemic time were older, more often diabetic, and hypertensive. The majority of patients presented directly to NUH (86%), with transfers from TTSH (10%) and AH (4%) making up the rest. Two-thirds of the patients (65%) arrived at the hospital during office hours and weekdays.

Cardiogenic shock was presented in 16 (7.7%) of patients. The culprit vessel resulting in acute STEMI consists of left anterior descending artery (57%), right coronary artery (34%) and circumflex artery (9%). Adjuvant therapeutics administered includes coronary stenting (97%), glycoprotein IIb/IIIa inhibitors (47%), thrombectomy device (40%) and distal protection device (10%).

The median symptom to reperfusion and door-to-balloon times were 3 hours 55 minutes and 110 minutes respectively. Only 3.6% of patients achieved time to reperfusion in less than 2 hours after symptom onset. 35% of patients achieved door-to-balloon times of <90 minutes.

Clinical Outcomes by Time-to-balloon

Mortality at 1 month and 6 months was 8.7% and 10.7% respectively, whereas MACE at 1 month and 6 months were 8.9% and 14.1% respectively. The mortality at 1 month was reduced to 4.8% if cardiogenic shock patients were excluded.

Mortality and MACE rate both consistently showed an escalating trend with longer symptom-to-balloon and door-to-balloon time. (Fig.2) However, all outcomes did not reach statistical significance. Longer symptom-to-balloon time was a significant predictor of MACE event at 1 month (OR 1.441, $p = 0.011$, CI 1.088-1.908) and 6 months (OR 1.184, $p = 0.048$, CI 1.001-1.400), but not mortality after adjusting for differences in baseline variables by multivariate analysis. Door-to-balloon time, however, did not demonstrate any significant effect on outcomes.

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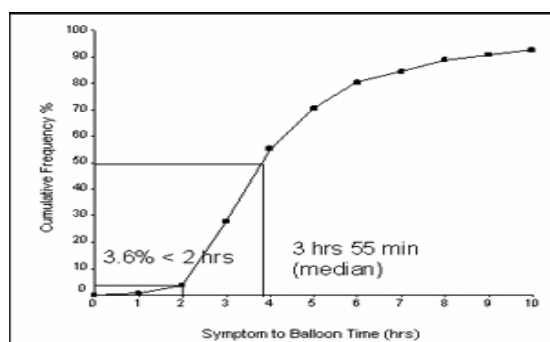
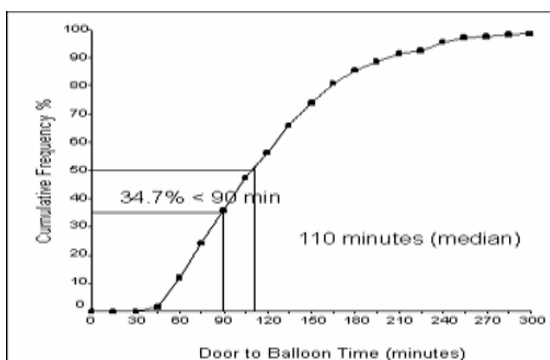


Figure 1. Cumulative frequency curves of Symptom to Balloon time. The median reperfusion time was 3 hours and 55 minutes, and 3.6% of patients achieved reperfusion in <2 hours.



Cumulative frequency curves of door to balloon time. The median door to balloon time was 110 minutes and 35% of patients achieved door to balloon times of <90 minutes

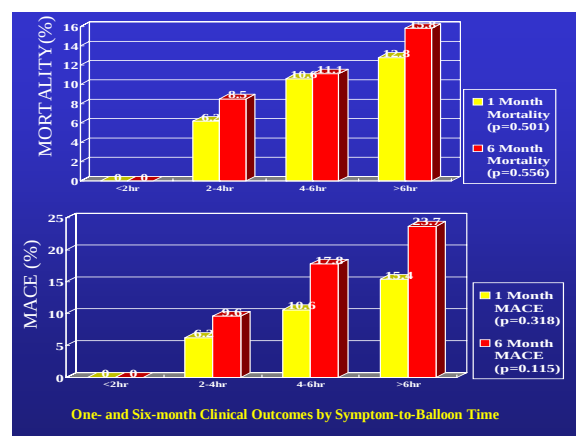
Discussion

The main finding of the present study is that among patients with STEMI undergoing modern mechanical reperfusion, delay in time from symptom onset to balloon is an important predictor of poor outcome. We are one of the first centres to report on this important issue in an Asian population.

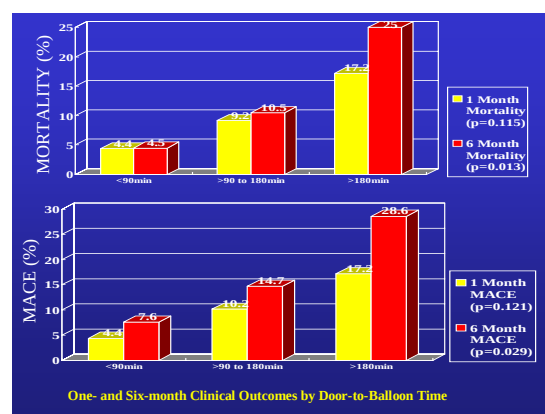
The association between increased duration of coronary vessel occlusion and degree of myocardial necrosis has been well-characterised in animal models.⁴ Therefore, late reperfusion is expected to result in poor flow, less myocardial salvage and thus suboptimal cardiovascular outcomes, even after optimal mechanical reperfusion. However, Zijlstra et al⁵ reported that mortality increased linearly with time delay only in patients treated with thrombolysis, whereas it was relatively stable in patients treated by primary angioplasty. Nevertheless, evidence is gradually mounting that time to reperfusion is just as important in primary angioplasty, as it is in thrombolytic therapy. In our cohort of 208 patients with STEMI undergoing primary angioplasty, our findings support the prognostic role of early restoration of myocardial perfusion.

The fact that only 3.6% of patients achieved a symptom-to-balloon time of less than 2 hours is alarming. Merely 35% of our patients achieved a door-to-balloon time of less than 90 minutes in accordance to the recommendation of American College of Cardiology/ American Heart Association (ACC/AHA) guidelines for the management of AMI.⁶ These findings imply that there are many opportunities for improvement in our current myocardial infarction management pathway.

Our finding that two-thirds of patients presented during office hours (Monday to Friday: 0800 h to 1700 h; Saturday: 0800 h to 1230 h) suggests the possibility that delay in presentation may have



One- and six-month Clinical Outcomes by Symptom-to-Balloon Time



One- and six-month Clinical Outcomes by Door-to-Balloon Time

Fig. 2. One- and six-month clinical outcome by symptom-to-balloon and door-to-balloon time.

occurred as a consequence of inaccessibility to medical facilities during after hours and on weekends. This factor could contribute to a considerable delay in the recognition of AMI.

Despite consistent increase in mortality and MACE with longer delay for symptom-to-balloon or door-to-balloon time (Fig. 2), statistical significance was not reached. These findings are most likely explained by the limitation of small sample size with low rate of mortality. Nonetheless, symptom-to-balloon time has been positively associated with MACE at both 1 month and 6 months.

Educating public and healthcare providers plays a paramount role in minimising delay at both times. Other emerging strategies include the administration of pharmacological agents to facilitate the opening of occluded arteries in transition to PCI ("facilitated" PCI).

Conclusion

Improving public awareness and accessibility of health services to patients with STEMI is essential in reducing poor outcomes. Our data suggest that physicians, hospitals, and healthcare systems should work together to reduce symptom-to-balloon time.

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Special Poster Presentation

Signal-averaged Electrocardiograms of Children with Ventricular Septal Defects Before and After Surgical Repair

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Introduction

Ventricular septal defect (VSD) is one of the most commonly encountered congenital heart defects in infants and children. The incidence of isolated VSD is about 2 per 1000 live births. While medical management is often the choice of treatment, surgical closure of the VSD may be indicated in some patients during the course of their disease. Operative mortality is often less than 5%.¹ Nevertheless, the incidence of ventricular dysrhythmias has been observed to increase with duration of postoperative follow-up.² The underlying mechanism(s) of such dysrhythmias have not been fully established.

The objective of this study was to assess the changes in signal-averaged ECG (SAECG) and the occurrence of late potentials (LP) in paediatric patients with ventricular septal defects, before and after corrective surgery. The hypothesis is that the presence of a VSD and/or surgical scarring of the myocardium may induce conduction disturbances that result in late potentials. These late potentials are high frequency, low amplitude potentials seen at the terminal portion of QRS and early ST segment of the ECG. Their presence may predispose an individual to ventricular dysrhythmias.³

Materials and Methods

Subjects

Paediatric patients with isolated VSD, either before (n = 148, 85 males, 63 females) or after (n = 66, 43 males, 23 females) corrective surgery were studied. Their ages ranged from 1 day to 17 years.

Age- and gender-matched healthy controls were similarly studied. The controls had no medical or cardiovascular disease by clinical examination and echocardiography.

All patients and controls had their SAECG measured. Relevant clinical variables including age, gender, height, weight, body surface area (BSA) and body mass index (BMI) were also measured.

Measurement of SAECG

SAECG parameters included 3 variables i.e., duration of filtered QRS (TQRS), duration of high frequency, low amplitude signals in the terminal portion of QRS complex that are <40µV (HFLA) and root mean square voltage of the last 40 ms of the filtered QRS (RMS₄₀). These were measured using a set of high-resolution ECG

equipment (MAC-15, Marquette Electronics, Milwaukee, Wisconsin, USA) and the Frank orthogonal XYZ lead system. Each subject was measured at rest and in a supine position. Surface ECG signals were processed for analysis of high-frequency, low-amplitude signals. Signal averaging reduced noise and improved the signal-to-noise ratio. A minimum of 200 cardiac cycles to a maximum of 600 cycles was averaged for each subject. The process of averaging was terminated when noise level was <0.3 µV or up to a maximum of 600 cardiac cycles, whichever occurred earlier. Signal-averaged QRS complexes were filtered with a spectral filter having a high-pass frequency of 40 Hz and a low-pass cut-off at 250 Hz. Determination of TQRS, HFLA and RMS₄₀ was done using the computer algorithm.

Age- and gender-specific criteria for defining LP was previously derived using a local population of healthy normal Chinese infants and children.⁴ LP was defined as present when an individual had 2 or more abnormal SAECG parameters present as defined by the age- and gender-specific criteria.

Statistical Analysis

Independent-samples T-tests were used to compare differences in SAECG and clinical variables between different groups.

Results

The median age of patients with preoperative and postoperative VSD was 7.2 months (range, 1 day to 15.6 years) and 40.4 months (range, 2.4 months to 17.4 years) respectively. 15.5% of preoperative and 28.6% of postoperative VSD children had right bundle branch block (RBBB). Among the postoperative VSD children, 19.7% had LP, in contrast to 2.7% of preoperative VSD children ($P < 0.001$) and 0.6% of controls.

SAECG and clinical variables of preoperative children were not different from that of controls but their BMI was significantly lower ($14.9 \pm 2.0 \text{ kg/m}^2$ versus $16.0 \pm 2.3 \text{ kg/m}^2$, $P < 0.0001$).

Postoperative VSD children had significantly prolonged TQRS and HFLA and lower RMS₄₀ when compared to age- and gender-matched controls (Table 1) while their weight, height and BMI were not significantly different. While postoperative VSD children were significantly older, they had significantly prolonged TQRS and HFLA and lower RMS₄₀ when compared to preoperative VSD

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Table 1. Comparison of Clinical Data, TQRS, HFLA and RMS₄₀ of Postoperative VSD Children and Controls

	Postoperative (n = 66)	Control (n = 66)
Height (cm)		
Mean ± SD	104.8 ± 33.0	106.7 ± 33.2
95% CI	96.7, 112.9	98.5, 114.9
Range	59.0-171.0	55.0-178.0
Weight (kg)		
Mean ± SD	20.3 ± 16.1	21.5 ± 15.3
95% CI	16.3, 24.3	17.7, 25.2
Range	5.0-67.9	5.5-72.0
BSA (m ²)		
Mean ± SD	0.75 ± 0.41	0.78 ± 0.40
95% CI	0.65, 0.85	0.68, 0.88
Range	0.27-1.75	0.27-1.86
BMI (kg/m ²)		
Mean ± SD	15.9 ± 3.0	16.8 ± 2.2
95% CI	15.2, 16.7	16.2, 17.3
Range	11.9-28.3	13.0-24.1
TQRS (ms)		
Mean ± SD	121.3 ± 22.8*	00.8 ± 8.1
95% CI	115.7, 126.9	198.8, 102.8
Range	86.0-175.0	82.0-127.0
HFLA (ms)		
Mean ± SD	24.4 ± 9.5*	21.4 ± 5.7
95% CI	22.1, 26.7	20.0, 22.8
Range	12.0-58.0	11.0-37.0
RMS ₄₀ (μV)		
Mean ± SD	63.9 ± 46.4*	89.7 ± 54.0
95% CI	52.5, 75.3	76.5, 103.0
Range	12.0-217.0	28.0-263.0

* Postoperative versus Control, *P* < 0.05

BSA: Body Surface Area

BMI: Body Mass Index

TQRS: Duration of filtered QRS

HFLA: Duration of high frequency, low amplitude signals in the terminal portion of QRS complex that are <40μV

RMS₄₀: Root mean square voltage of the last 40 ms of the filtered QRS

children (Table 2).

Discussion

The prevalence of LP in both the controls and preoperative VSD children are low, although the VSD children had slightly higher prevalence of LP (2.7%). Whether the presence of the structural cardiac defect, or the size and position of the VSD has any influence on the conducting pathways in the ventricular septum is not determined.

The prevalence of LP in VSD children after surgical correction of VSD is significantly higher (19.7%). Almost all these children had right ventriculotomy done during surgical repair of their VSD.

The presence of LP indicates slow, irregular or delayed propagation of electrical impulses in abnormal myocardium. As a consequence of such delayed activation or depolarisation of the myocardium high-frequency, low-amplitude micropotentials are seen at the terminal portion of the QRS complex and early ST segment of the ECG, thus giving rise to LP.³ It may be postulated that in patients after surgical correction of VSD, myocardial scarring and fibrosis may be present at the site of surgical incision. Such fibrotic tissue can give rise to barriers to impulse conduction, thus lengthening the excitation pathway. The higher prevalence of RBBB in our postoperative VSD children adds support to this postulation.

Various studies have indicated the prognostic and predictive value of LP for ventricular dysrhythmias in patients with myocardial infarction, sudden cardiac death and cardiomyopathy.^{5,6} The findings of this study imply that children after surgical correction of VSD and who had LP may be predisposed to ventricular dysrhythmias. The presence of myocardial scarring may be a contributory factor to an

Table 2. Comparison of Clinical data, TQRS, HFLA and RMS₄₀ of Preoperative and Postoperative VSD Children

	Preoperative (n = 148)	Postoperative (n = 66)
Age (years)		
Mean ± SD	2.5 ± 3.5	5.4 ± 5.0*
95% CI	1.9, 3.0	4.2, 6.7
Range	0.003-15.630	0.197-17.425
Height (cm)		
Mean ± SD	80.1 ± 31.0	104.8 ± 33.0*
95% CI	75.0, 85.1	96.7, 112.9
Range	46.5-163.0	59.0-171.0
Weight (kg)		
Mean ± SD	11.4 ± 10.3	20.3 ± 16.1*
95% CI	9.7, 13.0	16.3, 24.3
Range	2.3-72.8	5.0-67.9
BSA (m ²)		
Mean ± SD	0.48 ± 0.31	0.75 ± 0.41*
95% CI	0.43, 0.53	0.65, 0.85
Range	0.17-1.78	0.27-1.75
BMI (kg/m ²)		
Mean ± SD	14.9 ± 2.0	15.9 ± 3.0*
95% CI	14.6, 15.2	15.2, 16.7
Range	10.3-27.4	11.9-28.3
TQRS (ms)		
Mean ± SD	97.1 ± 11.5	121.3 ± 22.8*
95% CI	95.2, 99.0	115.7, 126.9
Range	70.0-148.0	86.0-175.0
HFLA (ms)		
Mean ± SD	20.0 ± 5.6	24.4 ± 9.5*
95% CI	19.0, 20.9	22.1, 26.7
Range	10.0-46.0	12.0-58.0
RMS ₄₀ (μV)		
Mean ± SD	137.7 ± 80.6	63.9 ± 46.4*
95% CI	124.6, 150.8	52.5, 75.3
Range	12.0-425.0	12.0-217.0

* Postoperative versus Preoperative, *P* < 0.05

BSA: Body Surface Area

BMI: Body Mass Index

TQRS: Duration of filtered QRS

HFLA: Duration of high frequency, low amplitude signals in the terminal portion of QRS complex that are <40μV

RMS₄₀: Root mean square voltage of the last 40 ms of the filtered QRS

increased risk of ventricular dysrhythmias.

In summary, it is noted that there is a higher prevalence of children have RBBB and LP after the surgical repair of VSD, as compared to preoperative VSD children. The presence of LP may predispose these children to ventricular dysrhythmias.

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Causes of Isolated Prolonged Activated Partial Thromboplastin Time (APTT) in an Acute General Hospital: A Guide to Fresh Frozen Plasma (FFP) Usage

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Introduction

Activated partial thromboplastin time (APTT) is a commonly requested coagulation test to assess the intrinsic pathway of coagulation. Fresh frozen plasma (FFP) has been used to correct prolonged APTT, often without further investigations. In our previous study, we found that about one-third of FFP requests were for inappropriate indications and a significant number of these requests were for the correction of prolonged coagulation tests, often without establishing the causes of the coagulation abnormalities.¹ We decided to investigate the causes of isolated prolonged APTT in an acute general hospital to further rationalise FFP usage.

Materials and Methods

Consecutive patients with prolonged APTT (>43 s) and normal prothrombin time (PT <15 s) in Tan Tock Seng Hospital between February 2002 and January 2004 were enrolled in the study after informed consent. Patients with prolonged TT were excluded from the study to exclude cases resulting from heparin contamination. The schema for investigation is shown in Figure 1.

APTT, PT and TT were performed using Actin FSL (Dade Behring, Marburg, Germany), STA CaCL₂ 0.025M, STA Neoplastine CI Plus, and STA Thrombin 2 (Diagnostica Stago, France) reagents respectively.

Lupus anticoagulant (LA) detection was based on the prolongation of lupus anticoagulant sensitive APTT (Diagnostica Stago, France), and correction by the addition of phospholipids. Two different assays were used: a dilute Russell's Viper Venom time-based assay with calculated ratio using reagent without and with phospholipids. A ratio of more than 1.2 was considered positive (Dade Behring, Germany). This was confirmed with a second test using hexagonal phase phosphatidylethanolamine as the source of phospholipid. A difference in APTT of more than 8 s with and without HPE was considered positive (StacLOT LA Kit, Diagnostica Stago, France).

Coagulant factor VIII (FVIII), IX, XI, and XII assays were APTT-based assays using factor-deficient plasma (Diagnostica Stago, France). Factor VIII inhibitor was quantified by mixing the test plasma with control plasma containing a known amount of FVIII. The level of inhibitor present was then calculated by comparing the residual FVIII activity of a patient-control mixture and a buffer-control mixture and expressed in Bethesda Units. Plasma Von Willebrand factor antigen (vWF:Ag) was quantified by immunoturbidimetric method (STA Liatest vWF, Diagnostica Stago, France).

All the above tests were performed using automated machine STA Compact except for StacLOT LA, which was performed using ST Art (both by Diagnostica Stago, France).

The causes of prolonged APTT were assigned according to the following criteria. If LA was positive, the cause was LA except when the test for specific FVIII inhibitor was positive, which would mean that the cause was FVIII inhibitor. Factor deficiency was diagnosed when factor VIII, IX, XI and XII levels were less than 41%, 42%, 56% and 44% respectively, based on the sensitivity of our APTT reagent. Probable von Willebrand disease (VWD) was assigned if

vWF:Ag level was less than 50% based on previously established laboratory reference range. Cause was classified as unknown if above criteria were not met.

Results

Two hundred and four samples were analysed, with 27 excluded due to prolonged TT, leaving 177 for the final analysis. The median age of the cohort was 52 years old (range, 13 to 104). The female and male ratio was 1:1.27. Racial distribution was as follows: 133 Chinese, 20 Malays, 16 Indians and 8 belonging to other races, hence representative of the general hospital population of Singapore.

The most common causes were LA and unknown (53.1% and 31.6% respectively) and factor deficiencies were rare (Fig. 1). Not all cases positive for LA had non-correctable APTT. Amongst the 56 with unknown causes, 40 had correctable APTT whilst 16 had non-correctable APTT after 50:50 plasma correction. APTT was considered correctable if it shortened to within 5 s of control APTT or it was shortened by more than 50% from the original APTT compared to control. In this group, APTT prolongation is usually mild and less than 1.5 times of the mean APTT.

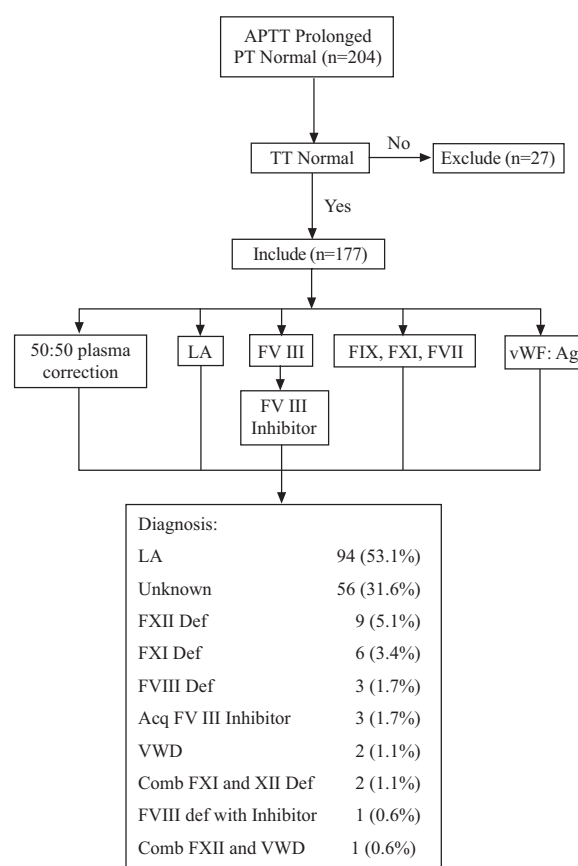
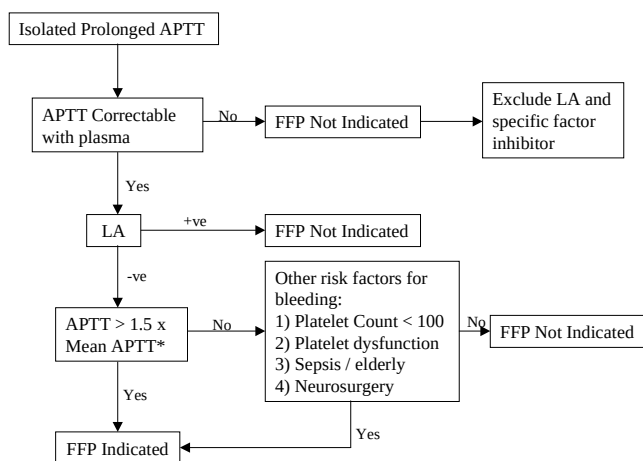


Fig. 1. Study schema and causes of isolated prolonged APTT detected.

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*Cut-off should be established after discussion with Surgeons and Anaesthetists

Fig. 2. Investigation algorithm for isolated prolonged APTT and decision for FFP usage.

Discussion

In our study, the most common cause of isolated prolonged APTT is LA, which predisposes patients to thrombosis, and FFP should not be used in these patients. The second most common group is those without detectable causes. The APTT is usually only mildly prolonged in this group and possible causes include inappropriate blood draw technique, deficiency of contact factors that are clinically insignificant or very weak LA where FFP is not indicated. Amongst the rest, only patients with Factor VIII, IX and XI deficiency may benefit from FFP treatment and these constitute a very small number. Even then, haemophilia A and B should preferably be treated with specific factor concentrates rather than FFP. This is also true for VWD. Factor XII deficiency is more often associated with thrombosis than with haemorrhage.² FVIII inhibitors require specific treatment and not FFP. Usually only factor XI level below its haemostatic level of 30% is associated with bleeding.³

In our cohort, this corresponds to APTT of more than 51 s, which is 1.5 times our laboratory's mean APTT. Most guidelines state that

FFP transfusion is indicated in bleeding patients or preoperatively only if APTT is more than 1.5 times the mean APTT.^{4,5} The basis may be that only APTT level above this level is associated with significantly low levels of coagulation factors. However, these general guidelines may not apply to patients who may have additional risk for excessive bleeding like thrombocytopenia.

Based on our findings, we have devised an algorithm for investigating isolated prolonged APTT and for the ordering of FFP to correct the coagulation defect (Fig. 2). Using this scheme, patients with FVIII inhibitor may sometimes be inappropriately given FFP but FVIII inhibitors usually arise in known haemophiliacs, who will be monitored for inhibitor development. They can also be acquired. In this situation, factor assays will usually be done in the setting of prolonged APTT (usually non-correctable with plasma). Patients with FVIII inhibitor should be recognised and managed accordingly to prevent uncontrollable bleeding.

Conclusion

Isolated prolonged APTT is usually due to causes that do not lead to increased bleeding. An investigational algorithm may help in determining the cause of the coagulation defect as well as in rationalising FFP use.

Acknowledgement

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Special Poster Presentation

MARS Liver Dialysis in Children with Acute Liver Failure

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Introduction

Molecular Adsorbents Recirculating System (MARSTM) has been shown to be an effective mode of liver dialysis in adults with acute liver failure (ALF) and acute-on-chronic liver failure. The MARSTM liver dialysis system is an albumin-filled circuit, which allows for the removal of both water-soluble and protein bound toxins such as

bilirubin, bile acids, aromatic amino acids and mercaptans from blood. Clinical studies over the last decade have demonstrated that the removal of these toxins is accompanied by a lightening of the degree of hepatic encephalopathy, as well as improvement in cardiovascular and renal functions.¹⁻³

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However, there is still currently, limited information on the safety or effectiveness of MARS™ in children.

We report our experience of MARS™ in 4 children with acute liver failure.

Materials and Methods

This is a retrospective chart review of children with acute liver failure who received MARS™ liver dialysis over the period 2001 to 2004 at the Children's Medical Institute, National University Hospital. Aetiologies of liver failure were: acute Wilson's Disease (2) acute Hepatitis B1 and acute liver failure of unknown aetiology (1) (Table 1). The weight of the children ranged from 18.6 kg to 55 kg. All the patients had been listed for liver transplant having met the King's College criteria for transplantation,⁴ and were being managed in the Paediatric Intensive Care Unit. All 4 patients were in grade 3 to 4 encephalopathy, and were mechanically ventilated and sedated. One patient was on intravenous inotropic support. Neurological status was assessed clinically. These included pupil size, reactivity and equality, blood pressure, plantar tone and reflexes. Liver function was monitored before and after each MARS™ session.

There was no modification of the circuit for these paediatric patients. Standard Baxter BM 25 and Gambro AK 100 renal dialysis machines with paediatric blood circuits were used, coupled with the MARS™ albumin circuit and monitor. No anticoagulation was employed.

Table 1. Demographic of Patients

Patient	Age (y)	Sex	Aetiology	Maximum PT (sec)
Pt.1	5	Male	Acute Wilson's disease	53.3
Pt.2	11	Female	Unknown, non A to E hepatitis	94.6
Pt.3	15	Male	Acute hepatitis B	81.4
Pt.4	6	Male	Acute Wilson's disease	67.1

PT: prothrombin time; sec: seconds

Results and Outcomes

A total of 9 sessions of liver dialysis were carried out were carried out between the 4 patients with an average of 2 sessions each, each lasting an average of 6 hours. All MARS™ dialysis sessions were uneventful and well-tolerated.

Three patients had normal Computer Tomography scans of the brain prior to MARS™ dialysis. This was mainly to exclude intracranial haemorrhage prior to liver transplant. Two out of these 3 patients had intravenous mannitol. All patients' pupils remained active throughout the dialysis sessions.

All 4 children had total bilirubin levels ranging from 282 mmol/L to 1247 mmol/L (mean = 615). There was significant fall in both conjugated bilirubin as well as total bilirubin. Reduction in conjugated bilirubin ranged from 21 mmol/L to 658 mmol/L with a mean of 176 mmol/L (SD = 231.54). The mean fall in total serum bilirubin was 160 mmol/L, with the average drop ranging from 4 mmol/L to 609 mmol/L (SD = 227.28) (Fig. 1). However, there were no significant changes in unconjugated bilirubin. In fact, there was some increase in the post-MARS™ results.

Maximum prothrombin time (PT) ranged from 53.3 to 94.6 seconds (mean = 74.1), a reflection of the severity of liver disease, and PT results did not change significantly with MARS™ treatments.

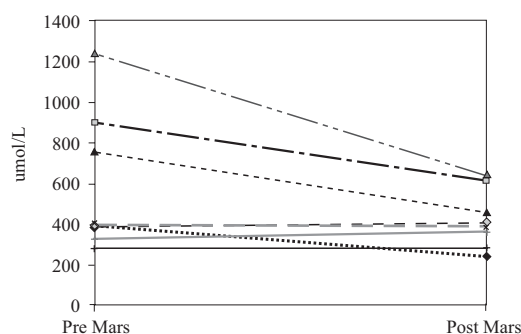


Fig. 1. Reduction in total bilirubin pre and post MARS™.

Two of the patients, 1 with Wilson's Disease, and the other with non A to E hepatitis received living related liver donors 3 days after the commencement of MARS™. Both received left lateral graft segments, the boy from his mother, and the girl from her father. The other 2 patients died 2 days after the commencement of MARS™ from liver failure, complicated by multi-organ failure. One had no compatible living related liver donor and the other deteriorated while a potential donor was being worked up.

Discussion

Despite new developments in paediatric intensive care management, the mortality of children with ALF remains high (50% to 90%, depending on the aetiology of disease).⁵ Mortality is usually related to complications such as cerebral oedema, sepsis and multiple organ failure. Urgent orthotopic or living related liver transplantation is currently the only effective treatment for these patients who are unlikely to recover spontaneously. However, liver transplantation is not always possible because of the shortage of organs or incompatible living related liver donors.

Artificial liver-assist devices such as MARS™ have the potential to prolong survival of patients with ALF; acting as a temporary support for the patient's liver function until the native liver recovers or as a bridging device until a liver graft becomes available.

Our experience has shown that MARS™ can be safely performed in children, and the efficacy in decreasing total serum bilirubin is similar to what is described in adult populations. In our local context, liver dialysis in children with acute liver failure may be useful in buying time for urgent assessment of a living donor.

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The Influence of Breast Feeding Compared to Formula Feeding on Infant Adiposity

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Introduction

Adult health may be influenced by foetal and early childhood nutrition. We postulate that breast milk or breastfeeding may have a beneficial effect on the growth pattern and body composition of infants, which may result in a lower incidence of obesity and its attendant complications later on in life.

Materials and Methods

We compared growth, skin-fold measurements and fat mass in breastfed (BF, n = 63) versus formula-fed (FF, n = 37) full-term infants, from birth until 12 months of age. The BF group was exclusively breastfed up to 2 months of age, while the FF group was predominantly or exclusively formula-fed. Anthropometry, skin-fold measurements at 4 different sites and percentage fat mass as determined by the Deuterium Oxide dilution technique for measuring total body water were performed at 3, 6 and 12 months of age.

Deuterium Oxide Dilution Technique

Deuterium is a stable non-radioactive isotope of hydrogen. It is accurate and non-invasive, similar to total body potassium (TBK) or total body electric conductivity (TOBEC), but unlike Dual-energy X-ray Absorptiometry (DXA). Total body water was calculated as 0.95 X dose/saliva concentration. The body fat percentage was then calculated from total body water using age-specific hydration factors of the fat-free mass and subtracting total body water from body weight. A pre-dose saliva sample (1 mL to 2 mL) was collected in a small plastic test tube about 2 hours before the next feeding. A weighed (accurate to 0.01 g) tracer dose of 0.2 g/kg (1 mL to 2 mL on average) deuterium oxide was fed orally to the infant and the tracer allowed to distribute evenly in the body water pool for 2 hours. A second saliva sample (1 mL to 2 mL) was collected after 2 hours. Saliva samples were stored in firmly closed test tubes at -20°C until analysis. After thawing and centrifuging at 2000 G, for 20 minutes, supernatants were pair-wise analysed using Fourier Transformed Infrared Spectroscopy (FTIR) and compared against weighed standards of deuterium oxide in water and 2 quality-control samples (low and high concentrations) after every 10 samples.

Results

All 100 babies were full-term at birth [(BF) 39.3 ± 0.9 weeks versus (FF) 38.8 ± 1.0 weeks]. Maternal characteristics such as ethnicity, age and body mass index, and the infants' sex ratio, weight, length, head circumference, body mass index and mid-arm circumference at birth were similar between the 2 groups. Solids were first introduced at 5.2 ± 1.1 (BF) versus 4.7 ± 0.9 (FF) months (*P* = 0.026). There were no significant differences between the 2 groups with regards to anthropometry, mid-arm circumference (MAC), skin-fold measurements, body mass index (BMI) or percentage fat mass (% Fat) at 3, 6 or 12 months as summarised (mean ± SD, *P* > 0.05) (Table 1).

Conclusion

There was a trend towards breastfed infants being leaner when compared to formula fed infants before and even after solids were introduced, although skin-fold measurements and body mass index showed the converse. If early nutrition has an effect on obesity later on in life, then exclusive breastfeeding for at least 2 months should be strongly advocated.

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Table 1

Age (mo)	Diet	MAC (cm)	Triceps (mm)	Bicep (mm)	Subscapular (mm)	Suprailiac (mm)	BMI	% Fat
3	BF	14.9 ± 1.9	9.1 ± 1.8	6.4 ± 1.3	8.4 ± 1.4	8.4 ± 2.1	17.0 ± 1.7	22.0 ± 6.5
	FF	14.2 ± 0.9	9.1 ± 1.7	6.9 ± 1.9	8.2 ± 1.5	8.1 ± 1.9	16.7 ± 1.9	22.7 ± 5.8
6	BF	15.1 ± 1.0	10.3 ± 2.2	6.1 ± 1.5	8.5 ± 1.9	7.3 ± 1.8	16.9 ± 1.7	22.8 ± 7.4
	FF	14.7 ± 1.1	9.1 ± 1.8	6.0 ± 1.4	8.6 ± 2.4	7.3 ± 1.6	16.8 ± 1.4	22.2 ± 5.2
12	BF	15.0 ± 0.9	8.6 ± 1.6	5.8 ± 0.9	8.4 ± 1.7	6.5 ± 1.2	16.5 ± 1.5	18.4 ± 2.7
	FF	15.0 ± 1.1	8.1 ± 1.3	5.8 ± 1.9	7.8 ± 1.9	6.8 ± 2.0	16.5 ± 1.1	19.1 ± 2.9

BF: breast fed; BMI: body mass index; FF: formula fed; MAC: mid-arm circumference

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Use of Complementary and Alternative Medicine in Paediatric Oncology Patients in Singapore

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Introduction

Complementary and alternative medicine (CAM) has been gaining acceptance worldwide.^{1,2} Although usage is thought to be widespread among paediatric cancer patients, local studies have not been done.

In our study, we aim to: (1) determine the prevalence of CAM usage before and after cancer diagnosis, (2) assess factors associated with CAM usage, (3) describe the reasons for usage or non-usage of CAM therapies, (4) estimate the costs incurred, and (5) assess parental perceptions towards CAM.

The results were anticipated to provide a profile of those parents likely to choose CAM therapies for their child, and then assist them to make their decision in an informed way.

Materials and Methods

Our study population comprised 73 paediatric cancer patients <15 years old (42 male children and 31 female children) from the Children's Cancer Centre of KK Women's and Children's Hospital, which treats two-thirds of paediatric oncology patients in Singapore. The primary caregiver completed an interviewer-administered questionnaire.

A pilot study, with standardisation of inquiry by interviewers and revision of the questionnaire, was done. The study proper was performed in May 2002. A subsequent telephone survey reached 59 of the original 73 caregivers (25 CAM users for cancer, and 34 non-users). This second phase focused on questions relating to spirituality as a treatment modality, benefits of CAM use, and overall satisfaction with the CAM therapy used, which were not sufficiently addressed in the original questionnaire.

Statistical Analysis

Data collected were analysed using SPSS v11.0. Descriptive and univariate analyses were performed to determine the factors associated with CAM usage. Bivariate analysis, including Pearson Chi-Square test was performed for categorical variables to determine if differences between each group were statistically significant.

Results

The demographics of the population interviewed were comparable to the Singapore Cancer Registry in terms of gender and race. Seventy-four per cent of the children were undergoing active treatment (either as inpatients or as day patients in the day therapy unit), with the remaining 26% on follow-up. 97.3% received chemotherapy, 15.0% received radiotherapy, and 13.7% received surgery. One received a bone marrow transplant.

Patterns of CAM Use

Forty-nine caregivers (67.1%) reported having used at least 1 CAM therapy since diagnosis of the child's cancer. The therapies most commonly instituted were changes in diet (55.1%), health supplements (44.9%), herbal tea (36.7%) and bird's nest (36.7%) ingestion (Fig. 1). Many respondents reported increased intake of fruits, vegetables or large amounts of fruit juice, and avoidance of

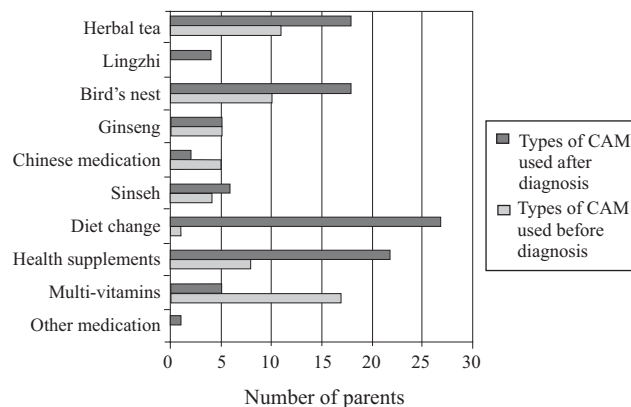


Fig. 1. Types of CAM used.

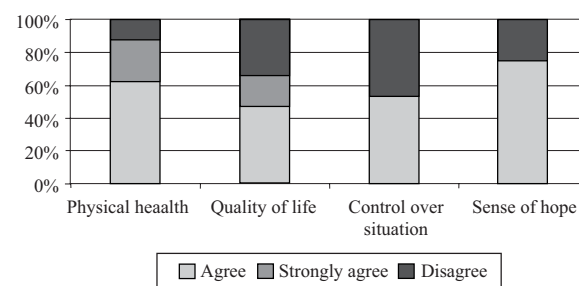


Fig. 2. Benefits of CAM used.

meat. One patient relied on "mushroom water" (drinking only water boiled with mushrooms). None adopted organic, macrobiotic or other unorthodox diets. Excluding dietary modification, 60.3% used at least 1 form of CAM. About 20 different kinds of health supplements were used, including cactus juice, noni juice, wheatgrass, plant extracts, pure wild honey, growth factors and enzymes. Herbal teas (like herbal soups, chrysanthemum tea and barley drink), regarded as "cooling" in traditional Chinese medical practice, were believed to decrease the "heatiness" of the body induced by chemotherapy. Only 6 patients (8.2%) consulted a traditional Chinese physician.

Phase II of the survey found that 25.4% of the 59 telephone respondents had used some form of spirituality, such as formal prayer (8 patients), laying on of hands (3), seeking help from a bomoh (2) or temple medium (1).

Predictors of CAM Use

Twenty-six of the 49 CAM users had used 1 or more forms of CAM since birth, the most popular being herbal tea, bird's nest and multivitamins.

Prior CAM usage (relative risk 1.93), Chinese race ($P < 0.001$),

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Buddhism/Taoism religion ($P < 0.001$) and dissatisfaction with conventional treatment were positive predictors. However, users and non-users did not differ significantly by household income, parental education, cancer type or conventional treatment modality used. Severe disease symptoms, treatment side effects or presence of cancer relapse were not associated with use.

Reasons and Expectations for CAM Use

Before diagnosis, the main reason for CAM use was to improve general health. Subsequently, most (85.7%) used it as an adjunct to conventional cancer treatments, with only 20.4% of respondents anticipating curative or anti-cancer effects. Other reasons cited were: to strengthen the immune system (63.3%) and to control chemotherapy side effects (25.0%).

Sources of Information

Most relied on more than 1 source, the most common being friends (51%), followed by other patients, then parent's own knowledge base. Eight per cent were recommended products by staff of health food stores.

Doctor-Patient Relationship

Although a majority (61.2%) felt it was important for the doctor to be aware of their CAM usage, only 44.9% informed the physician. Many parents felt that CAM remedies are derived from natural sources and are thus non-toxic. Some believed that the doctor would be unsupportive and discourage usage. On a positive note, 41.7% of non-CAM users followed advice from an oncologist and 87.5% of respondents were themselves wary of adverse interactions with chemotherapy.

93.9% of CAM users would continue such treatment even after completion of conventional therapy, with 65.3% recommending it to other parents.

Expenditure on CAM

Only 1 prior CAM user discontinued use after diagnosis. Overall, parents spent an average of \$197.90 per month. Prior CAM users spent an average of \$226 per month on their child, up from \$73.30. Despite the substantial costs incurred, many caregivers deemed it as money well spent.

Satisfaction with CAM and Conventional Treatment

The majority of CAM users (63.6%) agreed, with 24.2% strongly agreeing, that CAM improved their child's physical health and well-being (Fig. 2). Most (64.7%) felt their child's quality of life improved. CAM conferred psychological benefits of hope (32%) and a sense of control (50%). Overall satisfaction with both CAM (76.5% satisfied, 17.6% very satisfied) and conventional treatment (61% satisfied, 27.1% very satisfied) was high. Sixty per cent felt CAM was more easily obtainable and had fewer side effects than conventional treatment. However, CAM was not deemed to be safer, cheaper or more effective.

Discussion

The local prevalence rate of 67.1% is comparable to that previously reported in the literature.³ In Malaysia, Arrifin et al⁴ found that one-third of parents sought aid from traditional healers, with 13%

delaying conventional treatment. A Taiwanese study by Yeh CH et al⁵ found a CAM usage prevalence rate of 73%.

However, factors including parental education and poor prognosis were previously found to be predictive of use but we could not confirm this. The most common newly initiated therapies such as herbal teas or bird's nest are similar to those found by the Taiwanese study, probably because many of our patients are Chinese. The spectrum of CAM appears to be relatively narrow since therapies such as bioelectromagnetics, crystals and homeopathy were not popular locally. Parents within this small community may mutually influence each other's choice of CAM.

Our study highlighted the significant costs involved, which may constitute an unnecessary financial burden. Another point of concern is that 55.1% of patients were reluctant to disclose CAM usage to their doctors. The oncologist should initiate pre-emptive discussion in a non-judgmental manner. This may avoid disrupting the doctor-patient relationship and hopefully encourage compliance with conventional treatment.

Finally, though only 3 patients experienced ill effects with CAM, natural products are not necessarily safe or harmless. Of 260 traditional Chinese medicines investigated by Ko,⁶ 32% contained undeclared pharmaceuticals or heavy metals. Dietary changes and nutritional supplementation may affect tumour growth and drug bioavailability. Even antioxidants (vitamins C/E) can reduce chemotherapy effectiveness.

Conclusion

In conclusion, patients report physical and psychological benefits when they use CAM. Having a child with cancer is highly stressful for families. For parents disillusioned with the harsh treatments that may be necessary, CAM presents a supposedly non-toxic and holistic substitute. It is the argument to "leave no stone unturned"—to do everything possible—that may be hardest to refute.

It is foreseeable that CAM will have a growing impact on every facet of the healthcare system. Future research is thus important to clarify distinctions between potentially harmful alternative "cancer cures" and potentially beneficial complementary therapies employed as adjuncts to cancer treatment.

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Psychiatric Morbidity Among Emergency Department Doctors and Nurses after the SARS Outbreak

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Introduction

During the outbreak of Severe Acute Respiratory Syndrome (SARS) from 13 March to 31 May 2003, the study emergency department (ED) was designated as Singapore's national screening centre for SARS and was closed to all other patients. Overnight, the healthcare workers (HCW) of the study ED became the nation's front-liners in the battle against SARS, doing work and facing risks that were different from those of HCW in the rest of the hospital and in the ED of the other 5 public hospitals. To help HCW cope with the unusual stressors e.g., high rate of transmission among HCW, stigmatisation, taking care of sick colleagues, the study hospital and ED introduced psychosocial interventions.

Objectives

This study aimed to (1) determine the psychiatric morbidity among ED doctors and nurses 6 months after the outbreak, and (2) examine the coping strategies adopted by these doctors and nurses.

Materials and Methods

A self-administered questionnaire survey of ED doctors and nurses was conducted 6 months after the outbreak in November 2003. Inclusion criteria were all ED doctors and nurses who had patient contact at any time during the 80 days of the outbreak. The questionnaire was anonymous and participation was voluntary. Data collected included demographics and responses to these validated instruments (a) Impact of Event Scale (IES),¹ (b) General Health Questionnaire 28 (GHQ 28),² (c) General Health Questionnaire 12 (GHQ 12)² to measure psychiatric morbidity, and (d) Coping Orientation to Problems Experienced (COPE)³ to assess coping strategies. The hospital ethics committee approved this study.

The IES is a 15-item questionnaire. Based on the subject's response, each item was scored 0, 1, 3 or 5 giving a maximum score of 75, with higher scores reflecting higher impact. For this study, IES total score ≥ 26 was the chosen threshold for psychiatric morbidity. The GHQ 28 had 28 items and GHQ 12 had 12 items. Based on the subject's response, each item was scored 0 or 1, with a maximum score of 28 for GHQ 28 and 12 for GHQ 12. Psychiatric morbidity was defined as a score of ≥ 5 on GHQ 28 or ≥ 4 on GHQ 12.

COPE is a multidimensional inventory with 60 items, of which sets of 4 are grouped to form 15 conceptually distinct coping strategies. The 15 scales are in turn categorised as: (a) problem-focused coping, (b) emotion-focused coping, and (c) less-useful coping.³ Problem-focused and emotion-focused responses were considered more adaptive than less-useful coping.

Results

Thirty-eight out of 41 (92.7%) doctors and 58 out of 83 (69.9%) nurses responded. There was no difference between the doctors' mean age of 31.7 years (95% Confidence Interval [CI], 30.1-33) and that for the nurses at 32.1 years (95% CI, 29.7-34.6). The gender difference between doctors and nurses was highly significant ($P < 0.0001$), with 83.3% of doctors being men and 80.3% of nurses

being women (Table 1). On IES, 17.8% of respondents scored ≥ 26 , while on GHQ 28, 18.8% scored ≥ 5 , indicative of psychiatric morbidity. Doctors reported significantly less ($P = 0.03$) impact, scoring 12.2 (95% CI, 8.6-15.8) on IES compared to nurses scoring 17.6 (95% CI, 13.4-21.7). The GHQ 28 was significantly correlated with IES total score ($P = 0.04$).

Respondents reported a preference for problem-focused and emotion-focused i.e., adaptive coping measures and almost never used alcohol or drug to cope (Table 2). There was no difference between doctors and nurses in their use of adaptive or less-useful coping strategies. Compared to nurses, a significant number of doctors chose humour ($P < 0.0001$) as a coping response. Compared to other ethnic groups, a significant number of Filipino HCW turned to religion ($P < 0.0001$) as a coping response. On the GHQ 28, those with psychiatric morbidity scored significantly higher ($P = 0.002$) in less-useful coping compared to those without psychiatric morbidity. There was no correlation between age and the number of years in current profession with the above scores.

Discussion

In June 2002, 9 months before the SARS outbreak, the mental health of the study ED HCW was assessed with GHQ 28, which showed that 29.4% of 17 doctors and 33.3% of 24 nurses had minor psychiatric disorder. Six months after SARS, the rates of psychiatric morbidity were surprisingly low among the study ED doctors and nurses, compared to the pre-SARS baseline and 2 other SARS-related studies.^{4,5} The authors speculate that the SARS outbreak was such a life-defining event for the respondents that their perceptions of stress changed drastically.

Table 1. Demographic Characteristics of Emergency Department Doctors and Nurses

Characteristic	Doctors (n = 38)	Nurses (n = 58)	P value
Response rate	92.7%	69.9%	<0.0001
Male	65.8%	8.6%	<0.0001
Mean age (y) (95% Confidence interval, CI)	31.6 (30.1-33)	32.1 (29.7-34.6)	0.69
Mean no. of years in current profession (95% CI)	7 (5.6-8.3)	10 (7.5-12.4)	0.03
Marital status			0.03
Single	44.7%	63.8%	
Married	55.3%	31%	
Divorced/separated	0	5.2%	
Nationality			0.21
Singapore	70.3%	67.2%	
Philippines	16.2%	19%	
Malaysia	10.8%	5.2%	
China, India	2.7%	8.6%	
Religion			0.52
Christianity	60.5%	44.8%	
Buddhism	13.2%	20.7%	
No religion	13.2%	13.8%	
Islam	5.3%	15.5%	
Hinduism, other	7.8%	5.2%	

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Table 2. Median Scores in Coping Responses

	^a Problem-focused coping	^b Emotion-focused coping	^c Less-useful coping	Humour	Alcohol/Drug use
Professional groups					
Doctors (n = 38)	52	50	23	10	6
Nurses (n = 58)	49.5	49	23	6	4
P value	0.1	0.75	0.69	<0.0001	0.74
Ethnic groups					
Chinese (n = 64)	47.5	47	22	8	4
Filipino (n = 17)	52	55	25	8	4
Malay, Indian (n = 13)	50	52	21	7	4
P value	0.3	0.02	0.39	0.8	0.97
Psychiatric morbidity					
IES <26 (n = 76)	48	47	22	8	4
IES ≥26 (n = 17)	55	61	25	7	4
P value	0.001	<0.001	0.02	0.19	0.62
GHQ 28<5 (n = 77)	50	48.5	22	8	4
GHQ 28 ≥5 (n = 18)	52.5	51.5	29	8.5	4
P value	0.17	0.09	0.002	0.6	0.17

^a Problem-focused coping: sum of active coping, planning, seeking instrumental support, suppression of competing activities and restraint coping

^b Emotion-focused coping: sum of seeking emotional support, positive reinterpretation, acceptance, denial and turning to religion

^c Less-useful coping: sum of focus on and venting of emotion, mental disengagement and behavioural disengagement

Coping responses are influenced by 2 sets of factors: environmental factors and personal-internal characteristics. Many of the measures adopted by the hospital during the outbreak influenced the ED environment: (1) Intra-hospital communications e.g., daily emails from the CEO, daily updates by head of department, (2) communications with the community and public relations management e.g., meetings with and pronouncement of support from political, community and business leaders and (3) welfare and psychological interventions e.g., food and welfare gift packs donated by well-wishers, display of

thousands of tokens of appreciation and encouragement from the community. Certain conditions unique to the ED created opportunities for HCW to bond and cope better than expected: (a) all ED HCW report to the same head of department, (b) there was already a strong sense of identity as the ED team before the outbreak, (c) the ED HCW were relatively young and hence more receptive to changes, (d) the higher level of disaster awareness and preparedness helped with the crisis mode of operation during the outbreak and (e) the ED senior medical and nursing team personally sponsored in cash or kind departmental celebrations and activities to encourage and enable HCW to relax and have fun despite the seriousness of the outbreak. All these measures influenced the coping responses of ED HCW.

Conclusions

With a supportive hospital and departmental environment, respondents chose adaptive coping in response to the outbreak and reported low psychiatric morbidity. The trend was for nurses to report higher stress levels compared to the doctors, reaching statistical significance on IES but not on the GHQ. Doctors chose humour while Filipino chose religion as their preferred coping responses. Psychosocial interventions to help HCW need to take into account these preferences.

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Special Poster Presentation

Impact of Reversibility of No-reflow Phenomenon on 30-day Mortality Following Percutaneous Revascularisation for Acute Myocardial Infarction – Insights from a 1328-Patient Registry

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Introduction

Occurrence of no-reflow during percutaneous coronary intervention (PCI) is associated with a high incidence of death and adverse events.¹⁻³ The risk of no-reflow is particularly high in myocardial infarction (MI) interventions. Various vasodilators including verapamil, adenosine and nitroprusside have been used to treat no-reflow phenomenon. While most patients respond to these medications with restoration of normal antegrade coronary flow, some are refractory to the treatment and the final flow remains impaired. The impact of reversibility of the no-reflow on clinical outcomes is currently unclear.

In this study, we sought to determine whether reversal of the no-reflow is associated with improved survival compared with refractory no-reflow.

Materials and Methods

The PCI registry at the Singapore National University Hospital was used to gather patient data for this analysis. Patients who underwent PCI for MI at our institution from January 2000 to June 2004 were recruited for analysis. The diagnosis of MI was based on chest pain lasting ≥30 minutes, ST segment elevation ≥2 mm in at least

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2 contiguous ECG leads and a greater than 3-fold increase in serum creatine kinase (CK) levels.

All procedures were performed according to standard techniques, and the interventional strategy was left to the discretion of individual operator. All patients were on life-long aspirin administration and received a loading dose of clopidogrel (300 mg). All patients who received coronary stents received clopidogrel maintenance (75 mg/day) for >1 month.

No-reflow during the procedure was defined as Thrombolysis In Myocardial Infarction (TIMI) flow <3 in the absence of flow-limiting stenosis or thrombus or dissection that occurred after initial restoration of flow. All patients with no-reflow received intracoronary nitroglycerin in repeated 100 µg to 200 µg boluses at the time no-reflow was diagnosed. In addition, various drug therapies including adenosine, verapamil and nitroprusside or a combination of these drugs were administered at the discretion of the operator, according to the recommendation from the Society of Cardiac Angiography and Interventions.⁴

Our analysis stratified patients according to the occurrence and persistence of no-reflow during PCI: (1) Adequate reflow: without no-reflow occurrence; (2) Reversible no-reflow: no-reflow occurred, but final TIMI 3 flow restored after intracoronary medications; (3) Refractory no-reflow: no-reflow occurred and persisted, final TIMI flow <3 despite medications. Thirty-day mortality rates were determined for each group.

Results

A total of 1328 patients who underwent PCI for MI were analysed. Coronary stenting was performed in 92% of the patients. Among the study patients, no-reflow (including reversible and refractory) occurred in 135 patients (10.2%). Of the 135 patients with no-reflow, intracoronary verapamil, adenosine, nitroprusside or a combination of drugs was used to treat the no-reflow in 70.0%, 17.7%, 3.5% and 8.8% of cases respectively. Intracoronary medications successfully restored final TIMI 3 in 108 patients (80%, reversible no-reflow). The remaining 27 patients (20%) have final TIMI <3 (refractory no-reflow). The baseline demographic characteristics and angiographic details of the 3 groups are shown in the Table. After controlling for other factors, patients with higher baseline haemoglobin (OR 1.67; 95% CI, 1.06-2.62, $P = 0.0026$) and platelet (OR 1.01; 95% CI, 1.00-1.02, $P = 0.044$) levels were more likely to have refractory no-reflow.

The 30-day mortality of the adequate reflow, reversible no-reflow and refractory no-reflow groups are shown in Figure 1. We observed a higher mortality in the refractory no-reflow group. In comparison with patients with adequate reflow, occurrence of no-reflow (including reversible and refractory) had a higher 30-day mortality (4.1% versus 9.8%, OR 2.5; 95% CI, 1.26-5.04, $P = 0.007$). Among the patients with no-reflow occurred, reversible no-reflow was associated with a significantly lower 30-day mortality (3.3% versus 36.4%, OR 16.57; 95% CI, 3.92-70.08, $P < 0.001$) compared with the refractory no-reflow.

Discussion

The major finding of the present study is that in comparison with refractory no-reflow, reversible no-reflow is associated with a lower 30-day mortality.

No-reflow after PCI has been shown to be associated with worsened short- and long-term outcomes. In accordance to the previous reports, our study shows that the occurrence of no-reflow is associated with a >2-fold higher mortality. Although intracoronary medications can improve coronary flow, the effect on mortality is not clear. We found that reversible no-reflow is associated with a 10-fold lower 30-day

Table 1. Baseline and Lesion Characteristics of the Study Patients

Characteristics	Good flow (n = 1193)	Reversible no-reflow (n = 108)	Refractory no-reflow (n = 27)
Age (y)	55.68 (11.38)	55.06 (11.19)	58.08 (14.24)
Female sex	207 (17.4%)	19 (17.6%)	10 (37.0%)
Ethnicity			
Chinese	804 (67.4%)	68 (63.0%)	19 (70.4%)
Malay	181 (15.2%)	16 (14.8%)	4 (14.8%)
Indian	181 (15.2%)	21 (19.4%)	3 (11.1%)
Others	27 (2.3%)	3 (2.8%)	1 (3.7%)
Risk factors			
Hypertension	658 (55.2%)	53 (49.1%)	14 (51.9%)
Diabetes mellitus	346 (29.0%)	35 (32.4%)	9 (33.3%)
Hyperlipidaemia	669 (56.1%)	54 (50.0%)	12 (44.4%)
Family history	52 (4.4%)	1 (0.9%)	0 (0%)
Smoker	539 (45.2%)	38 (35.2%)	10 (37.0%)
Medical history			
Stroke	46 (3.9%)	4 (3.7%)	3 (11.1%)
Peripheral vascular disease	10 (0.8%)	0 (0%)	1 (3.7%)
COPD	1 (0.1%)	0 (0%)	0 (0%)
Renal failure	10 (0.8%)	0 (0%)	0 (0%)
Previous PCI	64 (5.4%)	6 (5.6%)	1 (3.7%)
Previous CABG	18 (1.5%)	1 (0.9%)	0 (0%)
Left ventricular function			
≥50%	518 (63.2%)	60 (69.0%)	8 (53.3%)
35% to 49%	251 (30.6%)	24 (27.6%)	6 (40.0%)
<35	51 (6.2%)	3 (3.4%)	1 (6.7%)
Baseline haemoglobin (g/dL)	13.86 (1.84)	13.91 (1.82)	13.78 (3.32)
Baseline platelet (x10 ⁹ /L)	260.91 (76.04)	251.30 (67.78)	284.33 (94.14)
Baseline creatinine (µmol/L)	99.21 (71.27)	93.90 (23.85)	109.48 (54.47)
Multi-vessel disease	214 (18.0%)	26 (24.5%)	3 (11.1%)
Glycoprotein IIb/IIIa inhibitors	226 (18.9%)	39 (36.1%)	12 (44.4%)
Target vessel			
LAD*	617 (51.7%)	64 (59.3%)	17 (63.0%)
LCx†	145 (12.2%)	9 (8.3%)	1 (3.7%)
RCA‡	417 (35.0%)	34 (31.5%)	9 (33.3%)
SVG§	8 (0.7%)	1 (0.9%)	0 (0%)
Left main	6 (0.5%)	0 (0%)	0 (0%)
AHA/ACC lesion type			
A/B1	247 (20.7%)	19 (17.6%)	2 (7.4%)
B2/C	945 (79.3%)	89 (82.4%)	25 (92.6%)
Eccentric lesion	897 (84.1%)	78 (85.7%)	20 (90.9%)
Thrombus-laden lesion	491 (41.2%)	59 (54.6%)	19 (70.4%)
Ostial lesion	37 (3.4%)	4 (3.8%)	1 (4.0%)
Irregular contour	380 (35.6%)	51 (50.0%)	11 (44.0%)
Angulated lesion	95 (8.9%)	5 (5.0%)	4 (16.0%)
Calcified lesion	77 (92.8%)	6 (5.8%)	0 (0%)
Bifurcation	170 (15.8%)	22 (21.0%)	4 (16.0%)
Tortuous lesion	46 (4.3%)	1 (1.0%)	1 (4.2%)
Baseline TIMI flow			
0-2	571 (48.5%)	65 (60.2%)	21 (77.8%)
3	607 (51.5%)	43 (39.8%)	6 (22.2%)
Final TIMI flow			
0-2	59 (5.0%)	0 (0%)	27 (100%)
3	1115 (95.0%)	108 (100%)	0 (0%)
Peak creatine kinase (U/L)	2223 (3225)	3052 (3028)	3337 (2791)
Peak CKMB (µg/L)	158 (190)	235 (189)	231 (187)

Values are expressed as number (relative percentage) or mean values ± SD

* left anterior descending artery; † left circumflex artery; ‡ right coronary artery;

§ saphenous vein graft

mortality compared with refractory no-reflow.

Recently, Resnic et al reported that despite improvement in antegrade flow, administration of verapamil or nitroprusside was not associated with improved in-hospital outcomes in patients with no-reflow.³ While our data show that reversible no-reflow is associated with better 30-day outcomes, these data are not conflicting. Although

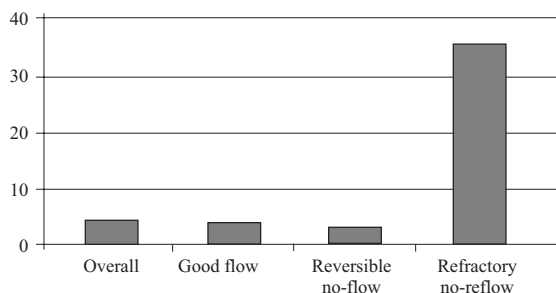


Fig. 1.

administration of vasodilators may not improve the clinical outcomes, it helps to separate the patients with better prognosis (reversible no-reflow) from those with worse prognosis (refractory no-reflow).

The 30-day mortality of patient with reversible no-reflow was found to be similar to those with adequate reflow in our study. In contrast, the PAMI investigators found that reversible no-reflow during PCI for MI was associated with a higher mortality compared with those without no-reflow.⁵ There are several fundamental differences in the 2 studies that may explain the different findings. Only stable patients that fulfill the stringent criteria were recruited in the PAMI trial while our registry has essentially no exclusion criteria and patients treated are those of 'real world' ones. No-reflow was defined as TIMI flow 0-1 in the PAMI study and 0-2 in our registry. The number of patients in the reversible no-reflow group in the PAMI was small (n = 16).

There are limitations in our study. We did not collect data on the myocardial blush scores (MBG) and TIMI frame count. These assessments are often time-consuming and require sophisticated equipment and trained personnel for their accuracy and reproducibility.

In contrast, epicardial TIMI flow can be reliably determined with visual inspection of angiograms by most interventional cardiologists during the procedure. Thus, unlike the previously mentioned techniques, identifying patients by visual angiography as having no-reflow during PCI allows for easy detection of a reversible versus refractory no-reflow.

In conclusion, our results suggest that the occurrence of no-reflow was associated with increased 30-day mortality. Among those with no-reflow occurred, reversible no-reflow was associated with a lower 30-day mortality compared with the refractory counterpart.

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Special Poster Presentation

Cardiovascular Abnormalities in Children on Long-term Dialysis: Analysis of Risk Factors

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Introduction

Cardiovascular disease is a major cause of mortality in adults with end-stage renal failure, accounting for 50% of all deaths.¹ Cardiovascular disease is also a significant cause of morbidity and mortality in children with end-stage renal failure on long-term dialysis. It accounts for 30% to 40% of all deaths in this group of patients.²

Left ventricular hypertrophy has been shown to be an independent risk factor for mortality. A report in 1992 stated that 30% of children on continuous ambulatory peritoneal dialysis and 22% of transplant children had left ventricular hypertrophy on echocardiographic examination. Another study in 2000 reported that 75% of their paediatric study group had left ventricular hypertrophy. In our local paediatric population, 34.5% of those with end-stage renal disease have severe left ventricular hypertrophy defined as LV mass index (LVMI) >51g/m².

This study examined potential risk factors that may affect cardiovascular status as measured on 2-dimensional echocardiography by left ventricular mass index (LVMI g/m²) and fractional shortening (FS%).

Materials and Methods

Data from 40 children with end-stage renal failure managed at our institution were retrospectively reviewed. Their mean age was 15.95 ± 6.33 years (range, 3.72-25.92 years) and they had undergone dialysis, mainly peritoneal dialysis, for 4.1 ± 2.9 years (range, 0.3-13.5 years). There were 15 males and 25 females. They had reached end-stage renal failure due to a variety of causes, including systemic lupus erythematosus, cystic dysplastic kidneys and reflux nephropathy.

The patients were evaluated for any change in their cardiovascular status based on the change in left ventricular mass index (ΔLVMI) and

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fractional shortening (Δ FS). These parameters were calculated based on echocardiographic measurements taken at the initiation of dialysis and at the most recent examination. We then analysed possible risk factors for cardiovascular disease, including age, race, sex, duration of dialysis (DUR), mean body mass index, mean systolic and diastolic blood pressures, mean haemoglobin, mean serum calcium (Ca), mean phosphate (Pi), mean intact parathyroid hormone and mean ferritin. Multivariate linear regression analysis was performed with either DLVMI or Δ FS as the dependent variables.

Results

The mean LVMI at baseline was $49.1 \pm 17.1 \text{ g/m}^2$ while the mean FS was $33.2 \pm 5.5\%$. At the end of the follow-up period, the LVMI had improved to $47.8 \pm 18.7 \text{ g/m}^2$ while the FS improved to $35.0 \pm 6.6\%$. The change in left ventricular mass index (Δ LVMI) over this duration ranged from -38.9 to 45.8 g/m^2 , (mean, $-1.33 \pm 19.10 \text{ g/m}^2$) whereas the change in ejection fraction (Δ FS) ranged from -10.7% to 23.8% (mean, $1.86 \pm 6.61\%$). Multivariate linear regression analysis showed that only serum phosphate ($P < 0.001$) was a significant predictor of Δ LVMI, whereas the serum calcium ($P = 0.004$) and dialysis duration ($P = 0.005$) were independent predictors of Δ FS. The linear regression equation for predicted DLVMI (g/m^2) = $26.52 \times \text{Pi} - 51.47$, while Δ FS (%) = $12.52 \times \text{Ca} + 0.89 \times \text{DUR} - 32.4$.

Discussion

Cardiovascular disease accounts for almost 50% of all deaths in dialysis patients. The incidence of cardiovascular death is much higher in dialysis patients compared to the normal population.

It has been increasingly recognised that abnormal calcium and phosphate metabolism play a part in the cardiovascular mortality and morbidity seen in patients with end-stage renal failure. Many studies have shown the association between elevated serum phosphate and cardiac deaths in patients on haemodialysis.³ An elevated phosphate or calcium-phosphate product is also an independent risk factor for all-cause mortality.⁴

Much attention has been placed on the association between calcium and phosphate metabolism and calcification of cardiac vessels and cardiac valves.⁵

The prevalence and extent of vascular calcifications are strong

predictors of cardiovascular and all cause mortality in haemodialysis patients.

There have been very few reports on the impact of calcium and phosphate metabolism on cardiac function as measured by left ventricular function. Rostand et al found a higher myocardial calcium content in patients on haemodialysis versus controls. The increased myocardial calcium was positively associated with an increase in the calcium-phosphate product and inversely associated with left ventricular ejection fraction.⁶ Similarly, in our study, we found that increasing serum calcium and phosphate influences the left ventricular structure, as measured by the left ventricular mass index, as well as left ventricular function, as measured by fractional shortening.

Surprisingly, the association between anaemia and left ventricular hypertrophy was not significant although it is now widely accepted that anaemia is an important factor in the development of left ventricular hypertrophy.

Our finding that there was a significant association between phosphate and the change in LVMI, and calcium and the change in fractional shortening, suggests that abnormal calcium and phosphate metabolism may affect left ventricular function. It also implies that good control of phosphate and calcium may help improve left ventricular function in children with renal failure on long-term dialysis.

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Special Poster Presentation

Umbilical Cord Blood Stem Cell from Unrelated Donors is a Feasible Alternate Stem Cell Source for Transplant in Patients with Genetic Diseases

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Introduction

Blood/marrow transplantation (BMT) using unaffected related matched sibling donors cures approximately 20% of patients with genetic blood and immunodeficiency disorders. The majority of patients, however, lack such donors. Transplantation using matched unrelated adult blood/marrow stem cell donors is an alternative but

is constrained by a limited racially appropriate donor pool, time disadvantage in the search process, high costs, and unacceptable risks of graft-versus-host disease (GVHD) when using un-manipulated stem cells, and rejection as well as infective risks when using manipulated stem cells.

To justify unrelated donor BMT, a relatively high-risk and high-

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cost procedure in patients with non-malignant diseases where safer, albeit non-curative supportive therapies are available, BMT needs to be safe and effective. To this end, focus has been placed on reduced intensity conditioning (RIC) to minimise regimen-related toxicity and the use of stem cells that are associated with lower risks of GVHD, such as umbilical cord blood (UCB) stem cells.

As the rejection risks with this strategy may be increased, novel regimens are required to improve the chance of success. In the last decade, there have been few anecdotal reports of successful unrelated UCB transplantation for patients with thalassaemia major (TM), fanconi anaemia (FA) and immunodeficiency disorders using conventional conditioning but none for patients with hyper-immunoglobulin M (hyper-IgM) syndrome. We report our experience with unrelated UCB transplantation for a small series of patients with genetic diseases such as these, who underwent both conventional as well as RIC regimens.

Materials and Methods

Over the last 6 years, 5 patients with genetic diseases [1 FA with myelodysplastic syndrome (MDS), 2 TM, 1 severe combined immunodeficiency (SCID), 1 hyper-IgM syndrome] received unrelated UCB transplantation at our institution. Except the 2 TM patients, the other 3 patients required emergent transplants due to deteriorating underlying conditions.

All patients were male with a median age of 5.5 (range, 0.5 to 17.0) years at transplant. All except 1 (patient with TM) received up to 2 antigens mismatched UCB grafts. UCB units were obtained from local (3 units) and overseas (3 units) banks. All except the 2 patients with immunodeficiency disorders (SCID and hyper-IgM syndrome) received conventional myeloablative conditioning.

The RIC regimens consisted of a combination of fludarabine with either intravenous cyclophosphamide or busulphan. The 17-year-old

patient with hyper-IgM syndrome received RIC with dual UCB units' transplantation. All patients received anti-thymocyte globulin (equine or rabbit) during conditioning. GVHD prophylaxis was with cyclosporine A and short-course methotrexate or mycophenolate mofetil. The median body weight of the patients at the time of transplant was 19.2 (range, 4.8 to 60) kg. The median total nucleated cell dose was 5.9 (range, 5.5 to 10.1) $\times 10^7$ /kg.

Results

Sixty per cent of the patients demonstrated myeloid engraftment at a median of 17 (range, 14 to 39) days. All engrafted patients achieved red blood cell and platelet engraftment within the first 100 days after transplant. At a median follow-up of 27 (range, 1.7 to 39.0) months, 80% of patients were surviving disease-free with documented donor chimerism in 3 of 4 patients. As of last follow-up, 2 of 3 patients had documented stable mixed chimerism while 1 (patient with TM) was 100% donor chimera by molecular studies.

There was 1 primary graft rejection with autologous reconstitution (patient with TM) and 1 early death secondary to infection (patient with FA and MDS). There was no regimen-related mortality. Only 1 patient (patient with TM) had grade 1 acute GVHD of the skin and none of the long-term survivors had chronic GVHD.

Conclusion

The results of unrelated UCB transplantation in this small series of patients with genetic diseases are encouraging. The conditioning regimen evolved over time with a focus on reducing the intensity of the conditioning regimen in later patients without compromising engraftment rates. The use of a novel strategy combining RIC and multiple unit UCB units to enhance cell dose without increasing risks of cross-rejection or GVHD is attractive, requires further study and holds a promise of safe and effective transplants for patients with genetic diseases.

Special Poster Presentation

Double Deletions of Glutathione S-transferase Genes (GSTM1 and GSTT1) Reduce the Risk of Early Relapse in Childhood B-lineage Acute Lymphoblastic Leukaemia (ALL)

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Introduction

The treatment of childhood acute lymphoblastic leukaemia (ALL), the most common form of childhood cancer, is one of the great success stories of medicine as locally >75% of children with ALL are long-term survivors. This has been achieved through increased intensity in therapy and improved scheduling of the same successful chemotherapeutic drugs in vogue for the last 20 years. However, despite improved chemotherapeutic regimens, 25% of patients relapse, and drug resistance remains the underlying cause.

Inherited differences in the metabolism and disposition of drugs due to the polymorphism in the genes that encode drug-metabolising enzymes, transporters, or targets can profoundly influence the

efficacy and toxicity of therapy, affecting therapeutic response.¹ Glutathione S-transferase (GST) is an interesting candidate gene to study as it is involved in the breakdown of many classes of chemotherapy drugs. Specifically, crucial anticancer drugs, like prednisolone, dexamethasone, cyclophosphamide and anthracyclines are substrates of GST.

GST conjugates glutathione to active cytotoxic drugs, rendering them inactive. There are several subfamilies of isoenzymes including GSTM1, GSTT1 and GSTP1. Homozygous deletions in the GSTM1 and GSTT1 gene produce null genotypes, resulting in no activities. These null genotypes will result in lower degradation of active drugs and hence increased efficacy or unfortunately increased toxicity. Similarly,

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polymorphisms in the GSTP1 gene have also resulted in lower enzyme activity.²

The impact of GST polymorphisms on outcome of childhood ALL has been studied in Western populations with disparate results. Specifically, the European BFM study group found that both GSTT1 and GSTM1 null genotypes reduce the risk of relapse but the St Jude and CCG investigators found no effect of GST genotypes on treatment outcome in childhood leukaemia.^{5,6}

In our study, we explore the impact of specific GST genotypes on early relapse (≤ 30 months), and relapse outcome in B-lineage ALL. We enrolled 98 B-lineage childhood ALL consisting of 41 relapsed patients [early relapse = 20 (≤ 30 months), late relapse = 21 (> 30 months)] and 57 patients who have achieved complete clinical remission (CCR) for at least 30 months. The patients were recruited from 2 centres, National University Hospital, Singapore, and University of Malaya Medical Centre, Malaysia.

Materials and Methods

Patients were treated in consecutive clinical trials: NUH-ALL and HK-ALL at the National University Hospital, Singapore, and UH-ALL studies at the University of Malaya Medical Centre, Malaysia. Criteria for risk-stratification were based on initial total white blood cell count (TWBC), cytogenetics and age. Patients with poor prognostic features: TWBC $\geq 100 \times 10^9/L$, cytogenetics: t(9;22), t(4;11), hypodiploid, and age < 2 years and > 10 years were considered as high risk. Treatment for the standard risk group in the ALL studies was almost similar. Patient exclusion criteria in the analysis includes 1) T-lineage ALL, 2) infant leukaemia, 3) induction failure, 4) toxic death, and 5) non-compliance. There were 63 males and 35 females, and the median age was 4.2 years.

DNA and Genotyping

DNA was extracted from BM aspirates using Trizol (Invitrogen) methodology. Genotyping for GSTT1, GSTM1 and GSTP1 polymorphisms were carried out by RFLP and PCR.³ Only GSTP1 codon105 polymorphism was analysed.

Statistical Analysis

Genotype frequencies in relapse and CCR were assessed with the Chi-square test. Survival estimates were based on Kaplan-Meier analysis and the Breslow statistic was used to compare survival distributions for the different composite genotypes. Relapse-free survival estimates were defined as time from date of diagnosis to relapse or CCR.

Results

The different GST genotypes detected in the 2 groups of patients are shown in Table 1. The effect of the different GST genotypes on relapse-free survival is shown in Figure 1. Among the 3 subfamilies of GST studied, M1 null has the highest protective value on patients from early recurrence of the disease (early relapse = 30% versus non-early relapse = 56%, $P = 0.046$). Double null GST genotype showed a significant protective value on early relapse as compared to both GSTM1 and GSTT1 present ($P = 0.0214$, Breslow). Although there was a trend towards improvement, double null GST genotype when compared to single null genotype on early relapse was not statistically significant.

No significant association of GSTP1 genotype on early relapse was observed in our study ($P = 0.115$).

Discussion

Patients vary widely in their responses to drug therapy, and functional polymorphisms in genes encoding enzymes, which affect drug efficacy, may underline these inter-individual differences. Efforts

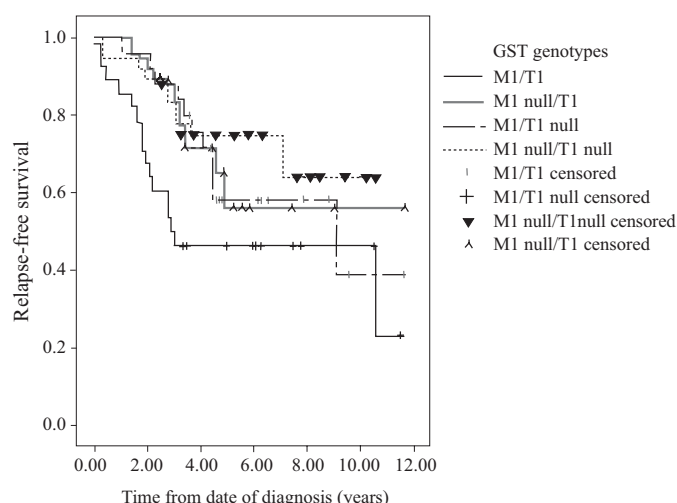


Fig. 1. The effect of the 4 different GST genotypes on relapse-free survival.

Table 1. Frequencies of the different GST genotypes in the 2 cohorts of patients: early relapse and non-early relapse. Note that the percentages may not add to 100% as some patients have deletion of more than 1 gene.

GST genotypes	Early ≤ 30 months (n = 20)	Non-early relapse CCR > 30 months (n = 78)
M1 and T1 present	11 (55%)	18 (23%)
Single null (M1 null or T1 null)	6 (30%)	38 (49%)
Double null (M1 null and T1 null)	3 (15%)	22 (28%)
M1 null	6 (30%)	44 (56%)
T1 null	6 (30%)	38 (49%)
P1 present	11 (55%)	53 (68%)
P1 homozygous	0 (0%)	6 (8%)
P1 heterozygous	9 (45%)	19 (24%)

have been made to gain closer insight into the role of these genes on both disease susceptibility and drug metabolism, and risk of relapse in childhood ALL.^{1,2}

We observed a significant impact of double null genotype (GSTM1 null/GSTT1 null) on early relapse and treatment outcome ($P = 0.0214$, Breslow) compared to GSTM1 and GSTT1 present. Double null GSTM1 and GSTT1 genotypes conferred a 40% reduction in risk of early relapse as compared to the presence of both GSTM1 and GSTT1 (Table 1). GSTM1 null has the highest protective value on patients from early relapse as compared to GSTT1 null, and GSTP1 polymorphism ($P = 0.046$). The impact of double null genotype (GSTM1 null/GSTT1 null) is greater than single null genotype (GSTM1 null or GSTT1 null) on early relapse and treatment outcome (Fig. 1). This may be explained in part by the role of GST in the detoxification pathway.

We studied children with B-lineage ALL, and patients who were non-compliant, failed to achieve remission in induction, had infant leukaemia and were below 1 year of age were excluded in the analysis. The excluded patients have other factors that are likely to impact on the outcome, like failure to take medications and very resistant disease that confounds the outcome despite the GST. We are currently prospectively collecting both toxicity and outcome data in our Singapore-Malaysia ALL trial and analysing this in the context of the patient's pharmacogenetic status. This study provides the basis for a larger prospective study. We hope that in the near future, individualised therapy based on a patient's pharmacogenetic make-

up can be tailored to improve cure rates, and this may warrant a high throughput screening to genotype multiple genes on a single platform.

Acknowledgement

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Special Poster Presentation

Evaluation of Particulate Respirators During the SARS Outbreak

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Introduction

Particulate respirators are designed to protect and minimise healthcare workers’ (HCWs) exposure to airborne pathogens. To be effective, they should fit different facial sizes and be properly fit-tested to prevent leakage. Prior to SARS, the 2 makes that were in use in Tan Tock Seng Hospital (TTSH) were 3M and Kimberly Clark. According to the TTSH Infection Control Unit’s fit test records, 65.8% of HCWs were fitted with the 3M 1860S (small size), while 26.5% were fitted with 3M 1860 (regular size). With the outbreak of SARS in Singapore, there was a drastic increase in demand for high-filtration masks and alternative makes of masks were sourced and evaluated. This presented a unique opportunity to test several different makes of masks for facial fit and leakage.

Materials and Methods

A qualitative fit test was used to assess the adequacy of respirator fit by observing HCW’s response to the test agent Bitrex. Bitrex is a bitter solution containing water, sodium chloride and denatonium benzoate manufactured by 3M. This solution is used in many consumer products as an aversive agent against ingestion, hence it is safe if swallowed. Sensitivity test and fit test were conducted using the 3M qualitative fit test apparatus. Three staff who wore “small” size and 3 staff who wore “normal” size (masks based on tests with conventional brand) participated in the evaluation. Staff had been advised not to eat, drink or chew sweets for at least 15 minutes before the test.

A sensitivity test was first done to ensure that the staff could detect the bitter taste of the solution at very low levels. The sensitivity test solution is a dilute version of the fit test solution. The staff were required to put on the hood and collar to contain the aerosol and instructed to breathe through his/her mouth while the sensitivity test aerosol was injected into the hood using a nebulizer. All the staff were able to detect the bitter taste of the solution.

The staff were given a few minutes to rinse their mouths with water to clear the taste. They were then instructed to don a respirator and

fit testing was repeated, this time with an undiluted fit test solution. After the initial injection of aerosol, the staff were asked to perform normal breathing, to turn their heads side-to-side and to nod their heads up and down. The staff were asked to count from one to ten as they performed the action. This was to detect any leakage when they talked. After that, the staff were instructed to take deep breaths. The test was terminated at any time the bitter taste of the aerosol was detected by the staff, as this would indicate leakage of the mask.

Staff who failed the first time were given a few minutes to rest

Table 1. Outcome of Mask Evaluation

Particulate respirators			
Brand	Model	Size	Number of staff who passed fit testing
1	A1	S	0
		M/L	3
2	A2	S	0
		M/L	0
	B1	S	0
		M/L	0
3	C	F	0
		M/L	0
4	D	F	0
		M/L	0
5	E	F	0
		M/L	0
6	F	F	0
		M/L	0
7	G	F	0
		M/L	0
8	H1	F	6
		M/L	0
9	I	F	0
		M/L	0
10	J	F	0
		M/L	0
11	K	F	0
		M/L	0
12	L	F	0
		M/L	0
13	M	F	1
		M/L	0
14	N	F	6
		M/L	0
15	O1	F	0
		M/L	0
16	O2	F	0
		M/L	0
17	P	F	1
		M/L	0
18	Q	S	0
		M/L	0

S: small; M: medium; L: large; F: free size

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and the fit test was repeated after redonning and readjusting the respirator. A second failure indicated that a different size or model respirator was needed. The test was successful and adequate respirator fit demonstrated if the entire test was completed without the staff detecting the bitter taste of the aerosolised solution. The process of fit test was repeated for the other makes of respirators.

Results

Twenty-one models of masks from 17 brands were evaluated (4 brands had 2 different models). Of these 21 models, 3 models of masks came in 2 sizes (small and medium/large), while 2 other models were available only in the small size. The other 16 models

were “free” sizes (Table 1). None of the HCWs who usually wear the “small” size could fit the 5 small-sized masks. Only 2 makes of the 16 free-sized masks could fit this group. The rest of the masks were either too big, or the nose metal piece too rigid to be moulded for proper fit.

Conclusion

The majority of the particulate respirators tested were not suitable for use in our sample of HCWs in TTSH. These results reinforce the need to evaluate different makes of masks, and are important for deciding which makes of masks to order should there be new outbreaks of airborne diseases.

Special Poster Presentation

Laparoscopic Radical Nephrectomy: Oncologic Outcome in 100 Cases

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Introduction

Until recently, open radical nephrectomy has been the standard of care for localised renal cell carcinoma. In 1990, Clayman and colleagues performed the first laparoscopic nephrectomy on a tumour-bearing kidney. This procedure has since become widely accepted in many centres throughout the world, and is fast becoming the new standard of care for localised renal cell carcinoma.

Materials and Methods

We retrospectively analysed 100 cases of clinical stage T1-T2 renal cell carcinoma treated by laparoscopic radical nephrectomy between October 1998 and July 2003 in a single institution (Westmead Hospital, New South Wales, Australia).

Preoperative imaging, operation reports, histopathology reports and follow-up information were studied.

Results

Fifty-nine male and 41 female patients with a median age of 61 years (range, 23 to 85) were included. The mean size of tumours was 4.6 cm (range, 2.0 to 10.0). Operating time ranged from 60 to 255 minutes, with a mean of 120 minutes.

There were 5 open conversions for the following reasons: obesity, dense peritoneal adhesions, tumour thrombus, hepatic vein injury and colonic injury.

Intraoperative complications included inferior vena cava injury (n = 1), splenic laceration (n = 1), hepatic vein injury (n = 1), colonic

injury (n = 1). There was no perioperative mortality. Postoperative morbidity included respiratory complication (n = 3), ileus (n = 2), pulmonary embolism (n = 1) and wound cellulites (n = 1).

Pathological stage distribution was as follows – T1a: 53 patients; T1b: 27 patients; T2: 9 patients; T3a: 6 patients; T3b: 5 patients. Histological cell type was as follows – conventional clear cell: 79 patients; papillary: 16 patients; chromophobe: 2 patients; collecting duct: 3 patients.

The mean follow-up duration was 24 months (range, 2 to 59). Five-year cancer-specific survival by Kaplan-Meier analysis was 91.6 % overall for all stages (T1a 96%, T1b 95.5%, T2 88.9%, T3a 80%, p3b 60%).

Discussion

In our experience with these 100 cases, we found laparoscopic radical nephrectomy to be technically feasible, and safe. Oncologic efficacy is at least equivalent to open radical nephrectomy. The 5-year cancer-specific survival of 91.6% overall compares favourably with recent published series (91% to 98%).

The advantages of faster postoperative recovery, decreased analgesic requirements, and shorter hospital stay have been shown in many published series.

We believe laparoscopic radical nephrectomy to be the new standard of care for localised renal cell carcinoma not amenable to nephron-sparing surgery.

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Aquaporin-4 is Correlated with Peri-tumoural Oedema in Meningiomas

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Introduction

Meningiomas are the most common non-glial primary intracranial tumours and they make up 15% to 20% of all primary brain tumours. They arise from specialised meningotheial cells mainly in arachnoid granulations or even develop from any meningeal structure or from ectopic cells rests of meningeal derivation. Significant cause of morbidity and death in meningiomas is contributed by tumour growth and compression of vital brain structures as well as peri-lesional brain oedema. Approximately 60% of meningiomas are associated with peri-lesional brain oedema. Several aspects such as age and gender of patient, size, location and grade of tumour have been evaluated in order to understand the pathophysiological mechanisms of oedema. However, at present the causative factors remain unclear.¹

Brain oedema is classified by Igor Klatzo into 2 main types: vasogenic and cytotoxic.² Vasogenic oedema involves the breakdown of blood-brain barrier (BBB) and extravasation of blood plasma into the brain parenchyma. Cytotoxic oedema is characterised by cellular swelling in the absence of any measurable breakdown of BBB.³ Central nervous system (CNS) neoplasm-induced oedema is an example of vasogenic oedema. Vasogenic oedema fluid is extracellular and accumulates primarily in white matter. Tight junctions in microvascular endothelia open in vasogenic oedema.⁴ Tight junctions are well-developed intercellular structures that form an impermeable seal between adjacent endothelial cells of blood vessels in the CNS. Tight junction proteins e.g., occludin, line the cytoplasmic face of intact tight junctions⁵ and establish the BBB.

Little is known about the regulation of water transport across the BBB, but it is well-recognised that the aquaporin family of water channel proteins is the major pathway by which water rapidly crosses cell membranes.⁶ The aquaporins are small (~30kDa) hydrophobic proteins that assemble in membranes as tetramers. Each monomer, consisting of 6 membrane-spanning tilted α -helical domains with cytoplasmically oriented amino and carboxy-termini, contains a distinct water pore.^{7,8} At least 10 aquaporins have been cloned. Aquaporin 4 (AQP4) is the predominant water channel in the brain.⁹ Within the brain, it is also expressed at the blood-brain and brain-cerebrospinal fluid interfaces.¹⁰ At the cellular level, AQP4 is expressed abundantly in a highly polarised distribution in ependymal cells and astroglial membranes facing capillaries and forming the glia limitans.¹¹ Recent studies have reported that AQP4 negatively influences the outcome from brain oedema¹² and suggested that AQP4 contributes to the development of brain oedema.^{13,14}

In this study, we investigate the role of tight junction proteins as well as AQP4 expression and its relationship to peri-tumoural oedema in human meningiomas.

Materials and Methods

After institutional ethics review and approval as well as informed consent, we studied 17 meningioma specimens after surgical resection. The specimens were predominantly skull base meningiomas excised by the senior author (IN). Ten of the tumours had severe peri-lesional oedema on MRI scans (T2 weighted images) while the other 7 tumours had no demonstrable oedema. Review of the MRIs was performed by

a member of the team blinded to the laboratory analysis (DNSL). Meningiomas were snap frozen in liquid nitrogen and stored at -80°C till analysis. An additional piece was placed in 4% paraformaldehyde for frozen section. A structurally normal cerebral cortex was obtained from a patient undergoing temporal resection to access a mesial temporal lobe lesion. All laboratory analysis was performed blinded to MRI data (TWL).

Immunohistochemistry

Immunoreactive occludin and AQP4 were detected by the labeled streptavidin-biotin method. Several contiguous 10 μ m sections were mounted on poly-L-Lysine-treated slides. One section was stained with haematoxylin and eosin. The remaining sections were extensively rinsed in PBS and endogenous peroxidases were quenched in 3% hydrogen peroxide for 5 minutes. After being washed in PBS, slides were incubated with primary antibody at room temperature for 2 hours with a 1:100 dilution of mouse anti-human occludin monoclonal antibody (Zymed Laboratories, CA, USA) or a 1:100 dilution of goat anti-human AQP4 polyclonal antibody (Santa Cruz Biotech Inc., CA, USA). Following 3 rinses with PBS, slides were then incubated with linking antibody (Dako) for 15 minutes, followed by 15 minutes with streptavidin-horseradish-peroxidase and then incubated for 8 minutes with the chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB; Dako). After, each incubation sample was rinsed 3 times with distilled water. Samples were counterstained with haematoxylin for 1 minute and nuclei blue in water. Slides were then dehydrated and mounted.

Western Blot analysis

Specimens were homogenised in triton lysis buffer containing 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% triton and 5 mM EDTA with protease inhibitor cocktail (Sigma-Aldrich, USA). Protein concentrations were determined by Bradford assay using bovine serum albumin as the standard control. 75 mg of protein was electrophoresed on a 12% SDS-PAGE gel and then electroblotted onto nitrocellulose membrane. The membrane was then blocked with 5% dried milk in Tris buffered saline with 1% Tween 20 and probed with 1:100 dilution of mouse anti-human occludin monoclonal antibody or 1:50 dilution of goat anti-human AQP4 polyclonal antibody. The membrane was then washed and incubated with horseradish-peroxidase (HRP)-conjugated goat anti-rabbit (Santa Cruz Biotech Inc., CA, USA) or rabbit anti-goat antibody (Chemicon International, CA, USA). Protein expression was detected with an enhanced chemiluminescence detection system (Pierce Biotechnology Inc., IL, USA). Bands were visualised on film and densitometry was used to estimate the relative amount of proteins.

Statistical Analysis

All statistics were performed with the SPSS software (Version 12; SPSS, Inc., Chicago, Illinois). Categorical variables were analysed using the Chi-square test. For means, a Student's *t*-test was used.

Results

Immunohistochemical staining

Alterations in BBB integrity via immunohistochemical staining of

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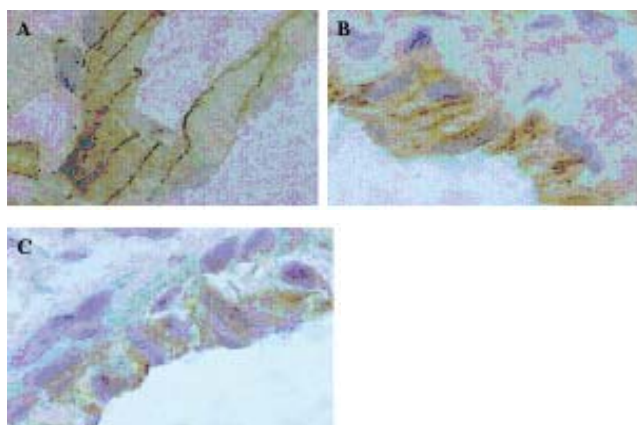


Fig. 1a. Immunohistochemical staining of the tight junction protein occludin in A. normal brain, B. oedematous meningioma and C. non-oedematous meningioma. Both groups of meningioma showed diffuse and fragmented staining of occludin.

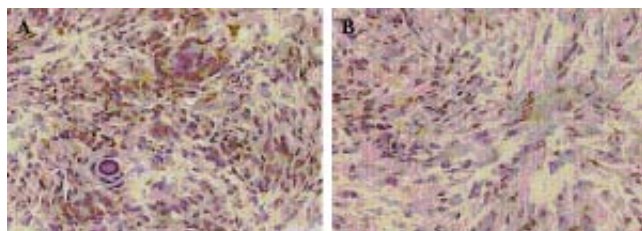


Fig. 1b. Immunohistochemical staining of AQP4 in A. oedematous meningioma and B. non-oedematous meningioma. AQP4 was upregulated in both groups of meningioma.

the tight junction protein occludin in the meningiomas showed significant disruption of BBB. It is demonstrated by fragmentation, absence or diffuse cytoplasmic localisation of DAB (brown staining) as compared to normal brain. However the degree of impairment of the BBB as observed under light microscopy was not significantly different between the meningiomas with marked oedema and no demonstrable oedema (Fig. 1a). Meningioma immunostained for AQP4 was not just restricted to the perimicrovessel region as observed in normal brain^{11,15,16}, but was upregulated throughout the specimens in both groups (Fig. 1b).

Protein Expression

Western blot analysis revealed that occludin protein level was not significantly different between the oedematous and non-oedematous meningiomas ($P = 0.28$) (Fig. 2a). However in AQP4 protein level, oedematous meningiomas had a significantly increased level of AQP4 expression compared with non-oedematous meningiomas ($217.2 \pm 94.8\%$ versus $74.4 \pm 63.1\%$, $P < 0.05$) (Fig. 2b).

Discussion

Cerebral oedema may result from a loss in structural integrity or from abnormalities of water homeostasis at the cellular level. As such we studied 2 areas; the integrity of a trans-membrane protein important in maintaining the structural integrity of the tight junctions and the expression of AQP4, a water channel recently implicated in cellular water homeostasis. In this study, we showed that AQP4 expression is upregulated in meningiomas and there is a significant correlation between AQP4 expression and the presence of oedema. Increased AQP4 expression suggests enhancement of oedema formation. The role of AQP4 in brain water homeostasis is still sparse but extrapolating the data from its function in the kidney, it functions

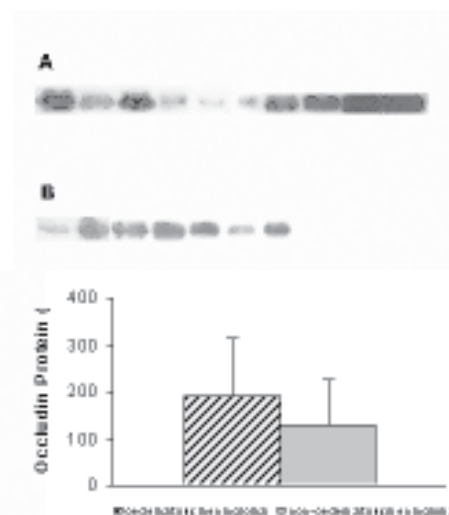


Fig. 2a. Expression of occludin protein (~59.1kDa) in (A) oedematous meningiomas and (B) non-oedematous meningiomas.

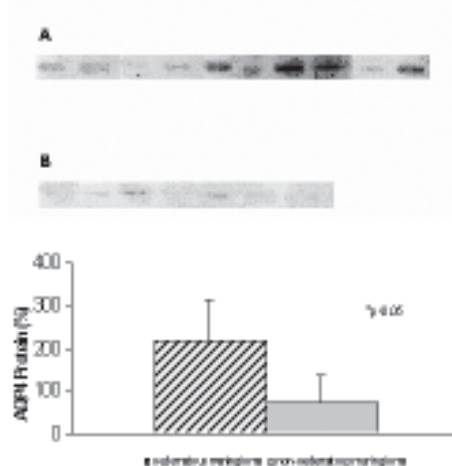


Fig. 2b. Expression of AQP4 protein (~34.8kDa) in (A) oedematous meningiomas and (B) non-oedematous meningiomas.

most likely by transporting water from the extracellular space into glial cells, protecting surrounding neurons from further osmotic stress.³ If the increase of AQP4 expression results in oedema formation and not oedema fluid clearance, its upregulation may be a maladaptive response, as is the case for upregulation of AQP2 in the kidney, in some fluid-retaining states.⁴ However it is shown in studies that AQP4-knockout mice showed reduced oedema formation after focal cerebral ischaemia and water intoxication.^{4,12,17} These studies provide evidence that AQP4 may be important in the formation of brain oedema.

The integrity of the BBB as observed in the immunohistochemical staining of occludin may have led to fluid leakage. However, the degree of disruption of occludin and its protein expression level were not significantly different between the oedematous and non-oedematous meningioma. Therefore BBB disruption and oedema formation may not be directly associated with tight junction pathology. However it has been shown by Vizuete et al that after neurological insult, striatal AQP4 mRNA was induced and it correlated with extravasation of Evans blue dye as a marker of BBB disruption and not with neuronal degeneration. Disruption of BBB could then have induced AQP4 expression to re-establish the brain osmotic equilibrium.¹⁸

This finding may be important as novel therapeutic targeting of aquaporin channels may limit oedema formation, which may greatly

ameliorate symptoms in non-operable cases where the symptoms may be due largely to oedema, greatly reduces the risk of surgery and aids in minimising the possibility of a stormy postoperative period from exacerbation of cerebral oedema.

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Special Poster Presentation

Estrogen Receptor Alpha Gene *PvuII* Polymorphism And Polycystic Ovary Syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common pathological causes of female infertility, affecting 5% to 10% of women in the reproductive age group. It is highly prevalent within families, suggesting a genetic basis. Several candidate genes have been studied as predisposing genetic factors contributing to PCOS, but neither of them has been widely accepted as a major cause for this syndrome.¹ PCOS is characterised by anovulation, excessive ovarian androgen production, and, consequently, infertility.

Estrogen receptors, ER α and ER β , mediate the physiological functions of estrogen. Estrogen not only acts as a key intraovarian modulator of ovarian activity but also exerts auto and paracrine roles on the follicle oocyte unit. It stimulates antral and pre-antral follicular growth.² In PCOS, the ovary is constantly exposed to estrogen and the multiple atretic and cystic follicles could result due to defective follicle protective action of estrogen.³

Several ER α gene polymorphisms have been identified, however *PvuII* polymorphism in particular has been found to be associated with a number of clinical conditions. *PvuII* polymorphism in ER α has been found to have a close association with breast cancer and spontaneous miscarriage.² Recently, an association between *PvuII* polymorphism and outcome of ovarian stimulation in

patients undergoing IVF has been reported.² In the present study an association of ER α gene *PvuII* polymorphism with PCOS was determined.

Materials and Methods

Study Subjects

One hundred and thirty four Singaporean Chinese female subjects having infertility due to PCOS were recruited in this study. Their ages ranged from 14 to 39 years (25.6 ± 6.71 years; mean $\pm$ SD). One hundred normal ovulatory Chinese women ranging between 18 and 44 years (32.7 ± 4.56 years; mean $\pm$ SD) with regular menstrual cycles (intervals between 23 and 39 days) were used as control subjects.

Investigation Protocols

Investigations included ovulation tests, hormone measurements, and abdominal ultrasonography and laparoscopy to confirm the polycystic nature of the ovaries. Plasma levels of E₂, FSH, LH and T were analysed by their specific radioimmunoassay using reagents provided by the WHO (WHO, Method Manual, 1981).

Determination of ER α *PvuII* Polymorphism

Genomic DNA was extracted from peripheral leucocytes by a standard procedure. DNA amplification was carried out using specific primers, ERpvuF:

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5'-CTGCCACCCTATCTGTATCTTTTCCTATTACAC-3'

ERpvuR:

5'-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3'

as previously described.² RFLP analysis was performed using *PvuII* as previously described.²

Statistical Analysis

The gene counting method determined gene frequencies. Statistical tests of significance and Chi-square and Fisher's exact test (2-sided) were carried out using SPSS for windows, version 12.0. One-way analysis of variance (ANOVA) was used for the statistical analysis of all serum values to achieve homogeneity of variance test. A *P* value <0.05 was considered statistically significant.

Results

The frequency of *PvuII* polymorphism was significantly higher in the patients (0.53) than controls (0.47) (*P* = 0.008, χ^2 = 9.676) (Table 1). The occurrence of homozygous *PvuII* polymorphism was also significantly higher in the patients (26.1%) than controls (11.0%) (*P* = 0.005) (Table 1). However, the occurrence of heterozygous polymorphism in patients was similar to that in controls.

Serum levels of E_2 was significantly higher in the homozygous group (355 pmol/L) than no polymorphism group (224 pmol/L) (*P* = 0.034), heterozygous group (238 pmol/L) (*P* = 0.020), and heterozygous and no polymorphism group taken together (233 pmol/L) (*P* = 0.001).

Serum levels of T and LH did not show any significant difference between the three genotypes ($0.436 \leq P \leq 0.999$). Serum level of FSH however, was significantly higher in the homozygous group (3.50 IU/L) than 2 other groups (2.57 IU/L) (*P* = 0.017) (Table 2).

Discussion

Various endocrine factors may contribute to the phenomenon of arrested follicular development, which is the hallmark of anovulatory infertility in PCOS. In PCOS, an increased recruitment of follicles or a decreased natural atresia occurs during stages of early folliculogenesis and development of early tertiary follicles. On the other hand atresia of tertiary follicles is accelerated compared with that in normal women and further follicle maturation is impeded.

Estrogen offers a folliculoprotective effect and plays an important role in recruiting or preventing atresia of follicles. In hypophysectomised rats, estrogen alone promotes preantral follicular development in the absence of endogenous gonadotropins.⁴ Androgens

when given systemically or produced locally through LH stimulation induce follicular atresia and administration of FSH prevents this effect.⁵ Thus it is possible that estrogen alone could be responsible for increasing recruitment or preventing atresia of primary, secondary and preantral follicles in women with PCOS.

ER α receptors have been demonstrated in ovarian epithelial cells, granulosa cells and theca cells of the ovarian follicles,² indicating importance of the receptor in the development of follicles. From a clinical perspective it is possible that a defect in the E-ER α binding system resulting from any mutation or polymorphism of the receptor protein would affect the normal function of the hormone.

In our study the incidence of *PvuII* polymorphism was higher in the patients than controls. The occurrence of homozygous *PvuII* polymorphism was also higher in the patients than controls. However, homozygosity in none of the groups showed any significance to patients with particular symptoms like acne, hirsutism or obesity. In our study, no statistical significance in serum levels of LH or T was seen. Serum levels of FSH were significantly higher in the homozygous group but were within the normal range. Nevertheless, serum levels of E_2 were found to be significantly higher in the homozygous group than no polymorphism group and heterozygous group. This could be attributed to the mechanism of estrogen resistance.

The *PvuII* polymorphism, though it does not result in amino acid change of the receptor protein, may co-segregate with mutations or regulatory sequence variations in the ER gene, which in turn may affect the ER expression or function.⁶ Furthermore, it has been reported that genes containing SNPs can cause different structural folds of mRNA, resulting in different biological functions that interact with other cellular components.

Conclusion

Therefore genetic variability of estrogen receptor gene through its estrogen resistance and interaction with environmental factors, and a small number of major causative genes including those involved in the paracrine and autocrine modifications of follicular growth, may contribute to the development of PCOS in some Singapore Chinese women.

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Table 1. Incidence of *PvuII* Genotypes in PCOS Patients and Controls

Subjects	Genotypes			Frequency of P	P value
	pp	Pp	PP	q	
Patients (134)	26	73	35	0.53	-
Controls (100)	17	72	11	0.47	0.008

P: *PvuII* positive; p: *PvuII* negative; pp: no polymorphism; PP: homozygotes; Pp: heterozygotes

Table 2. Hormonal Values of PCOS Patients in each Genotype

Variable	No polymorphism pp (n = 26)	Heterozygous Pp (n = 73)	Homozygous PP (n = 35)	Non-homozygous pp + Pp (n = 99)
E_2 * (pmol/L) (normal: 161-562)	224 $\pm$ 91	238 $\pm$ 106	355 $\pm$ 234 ^a	233 $\pm$ 101
T [†] (nmol/L) (normal: 3.1-11.4)	8.39 $\pm$ 3.10	8.51 $\pm$ 3.61	8.77 $\pm$ 3.48	8.51 $\pm$ 3.74
LH* (IU/L) (normal: 2.0-10.5)	1.61 $\pm$ 9.68	9.4 $\pm$ 8.48	11.95 $\pm$ 8.53	10.05 $\pm$ 8.85
FSH* (IU/L) (normal: 1.6-6.1)	3.20 $\pm$ 1.58	2.31 $\pm$ 1.45	3.50 $\pm$ 2.50 ^b	2.57 $\pm$ 1.53

P: *PvuII* positive; p: *PvuII* negative; pp: no polymorphism; PP: homozygotes; Pp: heterozygotes

Note: Values are given as means $\pm$ SD in SI units.

^a Significantly higher than pp group (*P* = 0.034), Pp group (*P* = 0.020) and these 2 groups taken together (*P* = 0.001)

^b Significantly higher than the other 2 groups taken together (*P* = 0.017)

* Measured at day 4

[†] Measured at day 21

Ultrarapid Prenatal Detection of Down Syndrome Using Real-time Multiplex Polymerase Chain Reaction (PCR) in Amniotic Fluid

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Introduction

Down syndrome (DS) or trisomy 21, affects 1 in 600 newborns. DS is the most common chromosomal aneuploidy causing severe mental retardation. In prenatal diagnosis, amniocentesis is the most common procedure for obtaining amniocytes for chromosomal analysis. Though reliable and accurate, 7 days to 21 days of culture for metaphase analysis are required before results are released. This extended period of waiting causes parental anxiety. Abnormal results are important in clinical decision-making, but normal results allay the anxiety associated with the longer wait for the full karyotype.

Alternative methods include analysis of uncultured amniocytes by fluorescence in situ hybridisation (FISH)¹ or by genomic DNA amplification and quantification.² In these tests, only common aneuploidies involving chromosomes 13, 18, 21, X and Y are analysed. FISH relies on visual counting of fluorescence signals within target foetal cells and is therefore labour-intensive, time-consuming and costly. The use of quantitative fluorescent-polymerase chain reaction (QF-PCR),³ to amplify and quantitate short tandem repeats reduces cost and time. However, a minimum of 6 hours is still required for QF-PCR.

Real-time PCR combines the amplification and quantitation steps in real-time. We hypothesise that by making use of real-time multiplex PCR, reporting time can be further reduced. In this study, we were successful in the detection of DS within 3 hours of amniocentesis with 100% accuracy.

Materials and Methods

Sample Collection and DNA Isolation

Sample collection for research was approved by the Institutional Review Board. Two millilitres of amniotic fluid, surplus to requirement for cell culture for conventional karyotyping were obtained after written informed consent from 85 mothers undergoing amniocentesis between 14⁺4 to 35⁺2 gestational weeks. The amniotic fluid was centrifuged to obtain the amniocytes pellet for resuspension in PBS. DNA was isolated from 200 µL of the amniocytes/PBS suspension using the QIAamp DNA Blood Mini Kit according to the manufacturer's recommendations (Qiagen GmbH, Germany).

Relative Quantitation of APP Against β-globin Gene Expression to Detect DS Using Real-time Multiplex PCR

Real-time multiplex PCR analysis was performed with the use of a PE Applied Biosystems 7000 Sequence Detector. For the detection of DS, amyloid gene (APP) was used as the target gene as it is located on chromosome 21. Endogenous reference gene used was β-globin. To examine the coamplification of both target and endogenous reference genes, it is essential that the probe of each gene was labelled with a different fluorescence dye to enable differentiation. In this study, APP probe was labeled with 6-FAM while β-globin probe is labeled with VIC. The sequence of APP and β-globin primers and probe combinations had been described.^{4,5} Commercial male and female genomic DNA were used as endogenous controls for the coamplification

of APP and β-globin in all runs.

Qualitative Measurement of APP/β-globin Real-time Multiplex PCR Efficiencies

Commercial male genomic DNA was serially diluted 5-fold and set as the standard. The standard curve is required to measure the efficiencies of APP/β-globin coamplification. Each sample and standard was run in triplicates with both sample and standards running in parallel.

Results

Qualitative Analysis and Relative Quantitation of APP Gene Expression

In all cases (100%), DS diagnostic results in this blinded study were concordant with their respective karyotypes. Both APP and β-globin amplified in all endogenous controls (100%). To calculate the mean δC_T (β-globin-APP) values, the difference between C_T of β-globin and APP in each well of the triplicate was averaged. This gives the mean δC_T (β-globin-APP) for relative quantitation of APP gene expression against β-globin. The δC_T (β-globin-APP) values were low (mean $\pm$ SD = -1.41 ± 0.17) in 82 pregnancies and high (mean $\pm$ SD = -0.75 ± 0.08) in 3 pregnancies (Table 1). With conventional karyotypes, these 3 cases with high δC_T (β-globin-APP) were confirmed to be DS while the remaining 82 were excluded of DS. The difference in the δC_T (β-globin-APP) values between normal and DS cases is significant ($P = 0.003$, Mann-Whitney rank-sum test) (Fig. 1).

Sensitivities of APP/β-globin Real-time Multiplex PCR Assay

Sensitivities of both APP and β-globin amplifications in this APP/β-globin real-time multiplex PCR assay are high. All standards

Table 1. Difference in the δC_T (β-globin-APP) between Normal and DS Amniotic Fluid Samples

	δC_T (β-globin-APP)	
	Normal	DS
Mean	-1.406	-0.753
Median	-1.40	-0.75
SD	± 0.17	± 0.08

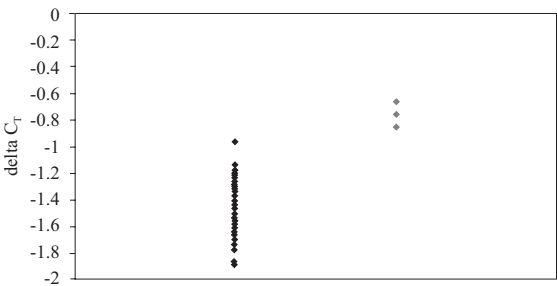


Fig. 1. Distinct differences in δC_T (β-globin-APP) between normal and DS amniotic fluid samples.

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including the lowest dilution of 15 GE/mL (GE: genome equivalents) were amplified in both *APP* and β -*globin* coamplification.

Standard Curves

Regression coefficients for all runs were at -0.99 (ideal -1.00). For *APP* standard curves, the mean $\pm$ SD slope was -3.68 ± 0.14 with the mean $\pm$ SD intercept of 39.95 ± 0.77 . For β -*globin* standard curves, the mean $\pm$ SD slope was -3.46 ± 0.08 with the mean $\pm$ SD intercept of 37.56 ± 0.45 .

Discussion

Real-time multiplex PCR amplifies both the target and endogenous reference genes in the same tube. Such coamplification minimises well-to-well variation and increases sample throughput. By using the mean δC_T (β -*globin*-*APP*), we were able to compare the gene expression level of *APP* and β -*globin*. This is known as relative quantitation of gene expression. We were accurate in detecting 3 DS out of 85 amniotic fluid samples.

Ideally, specific indication of the normality of the foetus should be available on the same day as the diagnostic invasive procedure. This would be possible if analysis results of uncultured amniocytes after invasive testing were ready within hours of the procedure. In our study, the time taken for DNA isolation, amplification and analysis

was within 3 hours of amniocentesis. We thus achieved our aim of ultrarapid diagnosis of DS.

We have demonstrated that real-time multiplex PCR of *APP* with β -*globin* as the endogenous reference in amniotic fluid samples is a rapid and reliable alternative technique to QF-PCR and FISH in prenatal diagnosis. This technique can be used to include other common foetal chromosomal aneuploidies like trisomy 18 (Edward's syndrome) and trisomy 13 (Patau's syndrome).

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Poster Presentation Abstracts – Day One

I/II – Day One/Day Two; xxx – Poster Board Number; AH – Allied Health/Health Sciences/Paramedical Disciplines; CPIP – Clinical Practice Improvement Programme; MPC – Medicine/Paediatrics (Clinical based); MPL – Medicine/Paediatrics (Laboratory based); NUR – Nursing; SC – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Clinical based); SL – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Laboratory based)
Layout of Posters: See Programme Guidebook pages 13-20

1001/AH

Outdoor Experiential Learning (OEL) in Healthcare for Team Development (TD) Programmes

S ANTHONY¹

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Aim: A study to determine the effectiveness of OEL over classroom methodologies for TD programmes for Nursing Officers (NO) was conducted in Tan Tock Seng Hospital.

Methods: Two groups (20 participants each) were exposed to OEL methodology involving low element activities as well as tunneling, which requires a high level of trust and support among team members and classroom based methodologies respectively. Both groups were subjected to the Team Development Inventory (TDI), a self-perception instrument, interjected prior to the programme and compared with 1 month after the programme. ANOVA of the difference in mean scores of both groups in each of the 10 parameters in the TDI was conducted. The key parameters were determined to be “Understand and Commitment to Goals” (UCG) and High Standards for Own Team’s Performance (STP).

Results: UCG revealed a t-test of 0.0001 and STP reveals a t-score of 0.0024. A statistically significant finding in both these key parameters indicates that the OEL methodology was superior in the context of Team Development Programmes. The “Law of Proximity” as defined by Wetheimer, supports this finding, in that through the use of appropriate metaphors and isomorphs, OEL programmes are able to create proximities between the activity, in this case tunneling, with actual applications in the workplace. Classroom methodologies tend to be theoretical and sometimes abstract.

Conclusion: OEL is a superior training methodology than classroom interventions in healthcare for Team Development Programmes, when used in conjunction with appropriate and relevant metaphors and isomorphs.

1002/AH

Prescription Drug (Benzodiazepine) Abuse/Dependence: Pattern of Use, Attitudes, Perception and Profile of CAMP Patients

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Aim: Recently, benzodiazepine (BZD) prescriptions in Singapore are arising (Kua & Tan, 1999). Doctors’ prescribing practice and doctor-shopping behaviour were found to contribute to BZD abuse (Boixet, Battie & Bolibar, 1996). This study aims to: 1) Examine the profile of CAMP patients with BZD abuse/dependence, 2) Investigate the relationship between the severity of BZD dependence and 2 key variables: doctor-shopping behaviour and patients’ perception on doctor’s knowledge, attitudes and practice.

Methods: Semi-structured clinical interview and medical records. Measurement: Bendep-SRQ, UT and BAL.

Results: The majority of an initial sample of 50 BZD dependent patients are married (40%), Chinese (90%), males (58%) in their late 30s. Half of them are currently unemployed. Two-thirds of them have used illicit drugs. BZDs were primarily obtained from local general practitioners (GPs) (74%). Most patients believe that doctors have insufficient knowledge (72%), low interest (72%) and poor practice (62%) in treating their BZD addiction. Patients’ perception of doctors’ attitudes and knowledge of BZD addiction were found to correlate positively with their severity of BZD problematic use ($P = 0.005$ and $P = 0.008$ respectively).

Conclusion: BZD abuse in Singapore is contributed to by both doctor-shopping behaviour and doctors’ prescribing practice. Doctors need training on the assessment and management of BZD dependence. Centralised control such as a national BZD registry may prevent doctor-shopping behaviour. Alternative coping strategies by early intervention programme and public education will be cost-effective. Future research will investigate knowledge, attitudes and practice among GPs.

1003/AH

Comparing the Spinal Block Profiles of Low Dose Racemic and Levo-Bupivacaine

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Aim: This pilot study was performed to compare the subarachnoid administration of low dose levobupivacaine and racemic bupivacaine in patients undergoing minor gynaecological procedures.

Methods: In this prospective, double-blind trial, 18 patients were randomised to receive either 5 mg hyperbaric levobupivacaine + 25 mcg fentanyl in 2.5 ml normal saline (Group LB) or 5 mg hyperbaric racemic bupivacaine + 25 mcg fentanyl in 2.5 ml normal saline (Group RB). Sensory block (loss of pinprick sensation), motor block (modified Bromage scale), analgesic characteristics and post-block complications were evaluated. Statistical analysis comprised the Student’s t-test and the Wilcoxon ranked sum test.

Results: Patient characteristics in the 2 groups were not statistically different. Sensory levels in both groups were adequate for surgery. Six (75%) patients in the LB group did not experience any motor block while the remaining 2 patients (25%) had full recovery of muscle strength (Bromage scale 0) within 30 minutes. In contrast, 8 (80%) patients in the RB group had a Bromage score of 2 or more. Only 2 (20%) patients in the RB group were able to perform a straight leg raise 60 minutes after intrathecal drug administration. Group LB also reported greater patient satisfaction scores that was significant. There was no difference in adverse effects between the 2 groups.

Conclusion: The intrathecal use of 5mg levobupivacaine with 25µg fentanyl achieved adequate anaesthesia for minor gynaecological procedures with less motor block and better patient satisfaction. It may be used to shorten recovery room stay and reduce costs.

1004/AH

Understanding the Basic Elements of Informed Consent: A Survey of Medical Professionals

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Aim: Informed consent is of paramount importance to the medical profession because of ethical and medico-legal issues. Our aim is to investigate the perceptions and practices of medical professionals in matters related to informed consent.

Methods: We used a questionnaire comprising 34 questions to survey 100 medical professionals over a 1-year study period on the 8 basic elements of informed consent. This includes methodology, risks, benefits, alternatives to treatment, confidentiality of data, compensation, contact information and voluntary participation.

Results: Our results showed that 17.4% of those surveyed fail to ensure patients fully understand the methodology of the trial, and 17.3% do not disclose the risks completely. 16.1% do not explain the benefits of the proposed treatment, with 17% not discussing the alternatives available. 29.8% fared poorly in assuring patients of confidentiality of records, while 17.1% fail to convey the idea that participation in the trial is voluntary. The worst performing areas were in the elements of compensation and contact information, where 51.9% do not inform patients that compensation is available in the event of a trial-related injury, while 51.3% do not provide patients with contact information for the trial.

Conclusion: In general, we find that there is a significant proportion of medical professionals with inadequate understanding of the basic elements of informed consent. There is therefore a strong need to train our doctors and research nurses in the proper way of taking informed consent.

1005/AH

Appearance of Multi-Drug Resistant Strains Among the Endemic Nosocomial Bacterial Flora at National University Hospital, Singapore

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Aim: The objective of this study was to analyse the type of Gram-negative bacteria causing nosocomial infections and their trend of resistance during the past 10 years (1994-2003). The antibiotic susceptibility pattern was compared with antibiotic use in the hospital.

Methods: The most common Gram-negative bacteria causing hospital

infections are *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Acinetobacter* spp. and *Pseudomonas aeruginosa*. The pathogens detected from clinical samples were identified by the Vitek-I automated system using GNI card, Microbact and API 20E Systems. Susceptibility testing was done by the Vitek-I system and Kirby-Bauer method. NCCLS criteria were adopted for interpretation of results. The results are saved in computer records.

Results: The effectiveness of major groups of antimicrobial agents; penicillins, cephalosporins, aminoglycosides, fluoroquinolones and penems has been decreasing steadily. The number of DDDs per 1000 patient days of the antibiotics studied has shown an increasing trend over the years. The major contributors to this increase are ciprofloxacin tablets and ceftriaxone injections. The incidence of ESBL producers among *Escherichia coli* (27%) and *Klebsiella* spp. (55%) too show an increasing trend.

Conclusion: The "best guess" empirical treatment is becoming increasingly difficult for clinicians. Introduction of techniques for the rapid detection of pathogens and their susceptibility patterns is becoming increasingly important. Recommendations on rational antibiotic therapy, rather than being generalised, should be based on evidence and local epidemiological factors. Antibiotic policies should be re-emphasised and updated according to the changing susceptibility pattern of bacteria.

1006/AH

Technical Evaluation of D Dual Kit on HbA1c and HbA2

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Aim: The Bio-Rad D-10 system is a point of care (POCT) high performance liquid chromatography (HPLC) instrument targeted at small and medium-sized laboratories. Features incorporated into the instrument include barcoding, interfacing, small footprint and primary tube sampling. The Bio-Rad D-10 Dual Kit is intended for the per cent determination of haemoglobins A2, F, A1c and abnormal haemoglobins.

Methods: We examined and compared the performance of the dual kit to our current Variant Classic method for HbA2 and Variant II for HbA1c.

Results: Using samples from our patients, the correlation obtained: HbA1c: D-10 = 1.13 (Variant II) - 0.95, $r = 0.99$, $n = 109$. The bias plot shows D-10 values to be >0.5 -1.4% HbA1c values $>10\%$. HbA2: D-10 = 1.01 (Variant Classic) + 0.21, $r = 0.99$, $n = 83$. The bias plot shows D-10 values to be >1.4 -6% at HbA2 values $>20\%$. HbE variants: D-10 = 1.27 (Variant Classic) - 4.18, $r = 0.77$, $n = 18$. Precision was evaluated using 2 levels of controls. Within day imprecision for HbA1c range of 4.9-11.3% ($n = 5$) obtained a coefficient of variation (CV) of $\leq 1\%$, between-day imprecision ($n = 8$) $\leq 2\%$ and total imprecision $\leq 2\%$ ($n = 12$). Within day imprecision for HbA2 range of 2.2-6.4% ($n = 5$) obtained a coefficient of variation (CV) of $\leq 2\%$, between-day imprecision ($n = 7$) $\leq 5\%$ and total imprecision $\leq 5\%$ ($n = 11$).

Conclusion: Our laboratory finds this instrument performs well in terms of precision and accuracy using samples frequently encountered in our hospital population. It is convenient, rapid and easy to use.

1007/AH

Indicator System for Herpes Simplex Virus Culture in National University Hospital, Singapore

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Aim: Conventional Herpes simplex virus cell culture includes monolayer cell culture and immuno-staining. Detection of viral growth is by presence of cytopathic effect (CPE). This takes up to 14 days for full investigation and the process is labour intensive and CPE dependent. Shell vial technique to demonstrate viral antigen by immunofluorescence test is a breakthrough to improve the time for detection; however, multiple culture sets must be inoculated to capture the full spectrum of viral growth. This is very costly and labour intensive. The indicator system is an in-house developed procedure established in National University Hospital, Microbiology laboratory which includes microtitre well and shell vial cultures. This protocol aims to shorten positive reporting time, cut cost and reduced labour and workload.

Methods: 1) Microtitre plate culture - Cell line selection - Cell line split ratio - Cell line maintenance 2) Shell vial culture - Cell line preparation - Time

schedule for immunofluorescence confirmation 3) Indicator system reading criteria - Proposed workflow

Results: 95% of positive results reported within 7 days. Exit immunofluorescence test results at 14 days incubation.

Conclusion: This method saves time, labour, cost and shortens reporting time.

1008/AH

Will the Compliance to the Measured Carbohydrate Exchange Diet at First Visit Be Used to Assess the Changes of HbA1c in Diabetic Nephropathy Patients?

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Aim: To investigate whether the compliance level to carbohydrate exchange and low refined sugar diet at first visit can be used to assess HbA1c changes in diabetic nephropathy patients.

Methods: 122 ($n = 122$) diabetic nephropathy patients (53% males, mean age 61 ± 9.96 years) had diet history taken by renal dietitians at the first outpatient visit (dated September 2002 to March 2004). The compliance of the diet was stratified into 3 groups (compliance, partial compliance and non-compliance) based on the carbohydrate exchanges and frequency of refined sugar consumption without acknowledging baseline HbA1c level. HbA1c at the baseline and post intervention (within 6 months) were compared.

Results: Eighty patients (66%, $n = 122$) had reduction in HbA1c. The initial assessment of dietary compliance level was correlated with baseline HbA1c level ($P < 0.05$). The means of baseline HbA1c for the compliance ($n = 47$), partial compliance ($n = 43$) and non-compliance ($n = 32$) groups were 8.9 ± 1.6 , 9.3 ± 1.3 and 9.6 ± 1.9 per cent respectively ($P < 0.05$). The rates of HbA1c reduction in these groups were 0.035, 0.058, and 0.02, respectively ($P = NS$).

Conclusion: The stratification of dietary compliance level using the developed criteria at first visit correlated with baseline HbA1c. However, this did not affect the changes of HbA1c. Subsequent follow-up will be required to evaluate the correlation between dietary compliance and HbA1c level. More comprehensive criteria of dietary compliance will have to be developed and validated.

1009/AH

C-reactive Protein: How Low Can You Go?

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Aim: C-reactive protein (CRP) is an established marker of inflammation and has recently been identified as a marker of cardiovascular risk. Its use has been complicated by the need for 2 assays: one with a wide dynamic range for traditional use and another with high sensitivity (low limit of detection) for cardiovascular risk assessment. This study evaluates a new CRP method that combines both features into a single assay.

Methods: The CRPLX (Roche Diagnostics) assay was run on the Roche Modular P analyser. Imprecision was assessed using QC material and data for the precision profile collected from on-board dilutions of the lowest control. Linearity was assessed using serum samples following NCCLS EP6-A. Accuracy at high levels was assessed against the Roche Tinaquant CRP assay and at <5 mg/L against the Roche CRP (Latex) HS assay.

Results: Imprecision CVs at 24 and 48 mg/L were 4.6 and 6.4% respectively. Functional sensitivity (CV 10%) was 0.7 mg/L with assay linearity to 250 mg/L. Deming linear regression showed CRPLX = $1.07 \times \text{Tinaquant} + 0.03$ ($n = 55$); mean bias 5.83 at high levels and CRPLX = $1.26 \times \text{CRPHS} - 0.04$ ($n = 57$); mean bias 0.14 at CRP <5 mg/L.

Conclusion: The new CRPLX assay shows good precision and accuracy with a wide dynamic range. It can be used for both high (traditional use) and low level (cardiovascular risk assessment) measurement. Its use will simplify laboratory processes while increasing the availability of CRP as a cardiovascular marker to clinicians and patients.

I010/AH**Ability of HIV Screens to Predict HIV Confirmation Results**R HAWKINS¹¹Department of Pathology and Laboratory, Tan Tock Seng Hospital, Singapore

Aim: Present policy requires reactive HIV screen results to be confirmed at the HIV Reference Laboratory. This study describes the delay in reporting of HIV confirmation samples and assesses the ability of the quantitative signal/cutoff (S/CO) data from the HIV screening test to predict the confirmation report.

Methods: Tan Tock Seng Hospital began using the Abbott HIV1/2 gO assay on the Abbott AxSYM for HIV screening in April 2001. All grey zone (S/CO 0.9-1.0) and reactive samples (S/CO >1.0) are sent for confirmatory testing. Anonymised details of samples from April 2001-2004 were entered into MS Access for statistical analysis, together with the final confirmation followup results for Indeterminate or Inconclusive confirmatory results.

Results: Three hundred and fifty-three samples were sent with a median turnaround time of 6 days (95% <10 days). The numbers of results returned as Negative, Indeterminate, Inconclusive and Positive for S/CO 0.9-3.8 were 26, 17, 1, 0 and for S/CO 6.7-80.0 were 0, 0, 9, 300 respectively. There were no samples with S/CO 3.9-6.6. When the results of follow-up of indeterminate and inconclusive cases are included, the numbers of final results returned as Negative, Positive and No followup for S/CO 0.9-3.8 were 36, 0, 8 and for S/CO 6.7-80.0 were 0, 305, 4 respectively.

Conclusion: The Abbott HIV1/2 gO AxSYM screening test S/CO ratio is an excellent predictor of the final confirmatory test result. This data should prove valuable for acute management decisions, such as needlestick injuries, while awaiting the results of confirmatory testing.

I011/AH**Genetic Characterisation of Renal Cell Carcinoma by Comparative Genomic Hybridisation CGH Initial Results with Three Cases**S ISHAK¹, SH LEONG², SY TAN¹, SJ CHIA³¹Department of Pathology and Laboratory, Tan Tock Seng Hospital, Singapore, ²DMS, National Cancer Centre, Singapore, ³Department of Surgery, Tan Tock Seng Hospital, Singapore

Aim: To study the genetic characterisation of renal cell carcinoma (RCC) by comparative genomic hybridisation (CGH).

Methods: Tumour DNA is labelled with FITC and normal DNA (derived from lymphocytes from a healthy volunteer) is labelled with Texas Red. Tumour and normal labelled DNA are allowed to hybridise to normal human metaphase preparations in a competitive fashion. The signals are detected and analysed using a computer fitted with an image analysis programme. The green to red fluorescence ratio measured along the chromosomal axis represents loss or gain of genetic material in the tumour at that specific locus.

Results: All 3 cases are sporadic RCC of clear cell type. Two cases showed deletions of 3p, in keeping with the known association with clear cell carcinoma of the kidney. In addition, 1 case showed -8, -10, -13q and -14q. Two other cases showed gains of 5q31qter, 8q24.1qter, 1q25q32 and 3q21. They also show losses of 10p11.2-13, 14q12, 17p13 and 4q12. Among the genes known to be associated with RCC at these loci include FHIT (3p14.2), DIRC2 (3q21), p53 (17p13) and TRC8/HRC1 (8q24.1). The only case showing loss of 17p13 (p53) is in a RCC of Fuhrman grade 3. The remaining 2 cases were graded as Fuhrman grade 1.

Conclusion: Whilst various translocations have been described in renal cell carcinomas, our initial findings suggest that copy number changes are present that may involve these same genes.

I012/AH**Accuracy Evaluation of Biorad D10 HbA1c Analyser**R HAWKINS¹¹Department of Pathology and Laboratory Medicine, Tan Tock Seng Hospital, Singapore

Aim: HbA1c measurement is the mainstay of diabetic monitoring and is available onsite at all National Healthcare Group (NHG) polyclinics. In 2003, all NHG polyclinics began use of the Biorad D10 HbA1c instrument. This study evaluates the accuracy of the D10 against the Roche Tinaquant

immunoassay system used in Tan Tock Seng Hospital Laboratory.

Methods: Over 5 days, 132 routine samples for HbA1c analysis (EDTA whole blood) were analysed using the Roche Tinaquant HbA1c assay (run on the Roche 917 analyser) and the Biorad D10 HbA1c analyser. The D10 is an automated HPLC system that delivers a HbA1c result in 3 minutes using 5 uL EDTA whole blood (dead space 1 mL).

Results: The bias plot showed a concentration dependent bias in D10 readings. The plot is an inverted U, with under-reading of ~0.5% at Roche HbA1c of 6% and 15%, equivalence at 7.5% and 13% and over-reading by up to ~1.0% at HbA1c of 10%. The imprecision of differences between the 2 methods increased above Roche HbA1c 9%. The mean bias (D10-Roche) was 0.18% (95% CI: 0.09-0.26) and the 95% limits of agreement were -0.80 to +1.15%. Clinical agreement between the 2 methods using MOH targets for ideal, optimal, suboptimal and unacceptable control was very good with a kappa coefficient of 0.95.

Conclusion: Despite obvious concentration dependent biases, the Biorad D10 showed good overall analytical and clinical agreement with the Roche Tinaquant assay. Nevertheless, clinicians should be aware that a difference of 0.5-1% between results from these 2 methods is not unusual.

I013/AH**Reducing Medication Errors in Nursing Homes Through Clinical Pharmacy Services**LL LOH¹, WM TENG²¹Department of Pharmacy, National Healthcare Group Pharmacy, Singapore,²Department of Pharmacy, Singapore

Aim: Elderly residents in nursing homes often face adverse drug events that may be preventable. Pharmacists carry out drug interventions through regular visits to minimise the occurrence. The objective of this study is to identify and evaluate the incidence of medication errors in 3 local nursing homes currently engaging the pharmacy services provided by National Healthcare Group (NHG) Pharmacy. These services include medication chart reviews and audits, accurate and timely supply of medications, maintenance of ward stocks, provision of continuing education lectures and drug information.

Methods: Medication charts were reviewed for a period of 6 months for 279 residents in Homes A and B, and a period of 3 months for 303 residents in Home C. All interventions made by the pharmacists within a specific time frame were collated according to each nursing home and subsequently classified into 2 main types of medication errors (prescribing and administration errors). The results obtained were categorised and analysed.

Results: The most common administration and prescribing errors for each nursing home were different drugs kept in the same container (21%) and therapeutic duplication (3%) for Home A, medications not served but not discontinued (26%) and inappropriate duration (13%) for Home B, and medications not served but not discontinued (70%) and inappropriate duration (3%) for Home C respectively.

Conclusion: Medication errors are common in the 3 nursing homes. Through the provision of clinical pharmacy services, most of them are prevented. Therefore, pharmacists can have a significant impact in reducing medication errors by identifying and resolving them.

I014/AH**Parenting Behaviour and Sense of Competence in Parents with Attention Deficit Hyperactivity Disorder (ADHD) Children**C KEE¹, J LEONG¹, J TEO²¹Department of Psychology, Institute of Mental Health/Woodbridge Hospital, Singapore, ²Department of Child and Adolescent Psychiatry, Institute of Mental Health/Woodbridge Hospital, Singapore

Aim: Studies suggest that parents with ADHD children have lower parenting self-esteem and exhibit more negative, coercive, over-reactive and lax parenting compared to parents of symptom-free children. There is less research that compares the parenting behaviour and experiences of parents with ADHD children against parents of children with other psychiatric or psychological conditions apart from ADHD. In order to determine if parenting differences were specific to parents of ADHD children or were common to parents who have children suffering from any psychiatric or psychological condition, this study compared parents of ADHD children against a community

sample as well as a non-ADHD clinical sample.

Methods: Thirty parents with children diagnosed as having ADHD were compared against 25 parents with non-ADHD children seen at a child psychiatric clinic, and 51 parents of children from a neighbourhood primary school. Questionnaires measuring parenting behaviour and parental sense of competence were administered. Comparison of means was made across the groups on the parenting variables.

Results: Findings suggest that parents with ADHD children exhibited significantly higher levels of overreactive parenting and were significantly lower in their sense of parenting efficacy compared to the community group of parents. However, they were not significantly different from parents of non-ADHD children who had other psychiatric or psychological problems on these parenting measures.

Conclusion: This study suggests that the parenting problems of parents with ADHD children are not unique to ADHD but common to parents of children with any psychiatric or psychological condition.

1015/AH

The Effect of the Presence or Absence of Flexor Digitorum Superficialis (FDS) of the Little Finger on the Grip Strength

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Aim: The purpose of this study is to examine the effect of the presence (independent or common) or absence of Flexor Digitorum Superficialis (FDS) of the little finger on the grip strength in a large general population.

Methods: Subjects with existing hand pathology were excluded from the study. This study evaluated 402 subjects (804 hands), 145 males and 257 females. The population age range was between 7 and 85 years. Using a standard test for detecting the presence of FDS, out of 804 hands, 48 were found to be FDS-absent, 306 FDS-common, and 450 FDS-independent. The Jamar Dynamometer was used to assess the grip strength.

Results: The average grip strength of those with FDS-independent was 31.69 kg. Those with FDS-common were 28.98 kg and those with FDS-absent were 28.49 kg. The results showed that those with FDS-independent had greater grip strength than those with FDS-common and FDS-absent, while FDS-common and FDS-absent groups had no significant difference in grip strength.

Conclusion: This study showed the effect of the presence of the FDS of the little finger on grip strength, it seems to reflect the importance of the FDS of the little finger in helping the individual achieve greater grip strength. It also showed the need to take into consideration the variable anatomy of the FDS of the little finger while evaluating population norm of the grip strength in Singapore population.

1016/AH

Irrational Beliefs and Job Stress Among Occupational Therapists in Singapore

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Aim: Studies over the years have looked into levels and sources of stress among occupational therapists. However, none has examined how beliefs systems affect job stress. This study attempted to investigate the relationship between intensity of irrational beliefs and stress levels. It also sought to examine associations between types of irrational beliefs and different sources of stress.

Methods: A cross-sectional survey was carried out between February and April 2003, during which self-reported questionnaires were posted or electronically mailed to occupational therapists working in various settings in Singapore. The 3 structured questionnaires were the Maslach Burnout Inventory, Sources of Stress in Occupational Therapy and Survey of Personal Beliefs. Thirty-seven occupational therapists responded.

Results: Results showed that intensity of irrational beliefs was unrelated to stress levels. However, certain types of irrational beliefs were related to the experience of certain sources of stress. In particular, low frustration tolerance was associated with stress regarding patient contact. Besides that, irrationality

in self worth was associated with stress pertaining to rewards and recognition as well as to professional value.

Conclusion: This study had shown that core beliefs may influence the experience of certain sources of stress. In order to help occupational therapists manage their stress better, cognitive strategies need to be utilised to work on the associated irrational beliefs. This will be cost-saving in the long run as it can help reduce attrition and stress-related illnesses.

1017/AH

Does the Burden of Caring for Patients with Schizophrenia Increase or Decrease with Prolonged Caregiving?

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Aim: The caregiving for patients with schizophrenia is a long term commitment. As community policies increasingly shift the responsibility of care to the families, the determination of caregiving burden becomes important. The aim of this study is to compare the short-term and long-term caregivers of these patients to ascertain if the burden increases or decreases with prolonged caregiving.

Methods: Caregivers of schizophrenic patients seen at IMH were interviewed using a questionnaire covering demographics and containing instruments to measure psychological distress and caregiving burden. A comparison was made between the 2 groups of caregivers. For mean comparisons, independent 2 sample t tests were used while for comparing percentages, Chi-square was used.

Results: One hundred and ninety-eight caregivers were surveyed, 81 were short-term caregivers and 117 were long-term. Some differences were observed. A higher percentage of the long-term caregivers were females, 65.8% compared to 51.9% ($P < 0.047$).

Conclusion: The findings showed that the short-term caregivers were more vulnerable and that with prolonged caregiving, the burden decreases. These findings are useful for service planning.

1018/AH

The Efficiency of Total Contact Inlays in Reducing Plantar Force in Neuropathic Ulcers

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Aim: The total contact inlay (TCI) is a full sock device that reduces pressure over prominent areas and redistributes it evenly over the plantar aspect of the foot. This study aims to ascertain the effectiveness of TCIs in reducing plantar force under neuropathic foot ulcers in order to aid healing.

Methods: A random sample of 19 subjects was taken from patients attending the podiatric clinic at Tan Tock Seng Hospital. The criteria for selection was presence of neuropathy, controlled underlying systemic pathology, good vascularity and presence of plantar neuropathic ulcer graded 2 and below for more than 6 months. Those subjects placed in the Study group were fitted with TCIs while those in the Control group wore their own footwear. They were given podiatric wound care at regular intervals and were followed up over 6 visits. The contact force under the ulcer was measured with the Economic Loading Force system manufactured by Tekscan and the ulcer size was assessed using the Optimas software system.

Results: The results show that the TCI reduces the maximum contact force by 58.28% while normal footwear only 10.07%.

Conclusion: The TCI is effective in reducing plantar force under neuropathic foot ulcers ($P < 0.05$). The ulcer-healing rate and the length of time the subjects wore the TCI showed a positive correlation ($r = 0.898$) suggesting that the longer the subjects wore the TCIs, the faster their ulcers healed.

1019/AH

Dietary Carbohydrate Exchanges and Glycaemic Control of Diabetic Patients – A Prospective Study on the Effect of Dietary Counselling

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Aim: To study the effect of compliance to a controlled carbohydrate exchanges (CCE) with low content of refined sugars diet; on changes in HbA1c in patients with diabetic nephropathy and poor blood sugar control.

Methods: Diabetic patients were selected from a renal clinic. Patients were entered into the study if their HbA1c was above 8.0%; and assessed to be non fully compliant with a CCE diet by a dietitian. After counselling, and 6 months of follow-up, the same dietitian reassessed their compliance to the CCE diet. The changes in compliance were correlated with the changes in the HbA1c. Thirty patients were entered into the study (57% were males). Mean age ($\pm$ SD) was 61 ($\pm$ 9.2) years.

Results: Thirteen patients (43%) improved on their compliance to the CCE diet. Of these, 10/13 (77%) patients had an improvement in HbA1c. Conversely, of the 17 patients who had no improvement in compliance, only 6/17 (35%) patients had an improvement in HbA1c ($P < 0.005$).

Conclusion: Counselling resulted in improvement of compliance to a CCE diet in nearly half of the patients on follow-up. This improvement was associated with a significant improvement in glycaemic control in patients with diabetic nephropathy and poor blood sugar control.

1020/AH

Neuronal Differentiation of Bone Marrow Stromal Cells

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Aim: Recent studies suggest that bone marrow stromal cells (BMSCs) can be differentiated into neuronal cells such as neurons, oligodendrocytes and astrocytes. However, these cells only exist in a small fraction out of the whole total bone marrow cells. This study was designed to isolate these cells and to differentiate them into neurons.

Methods: We cultured and isolated a sub population of mouse BMSCs simply referred to as flat BMSCs with regard to its morphology. These flat BMSCs were further expanded and differentiated using different chemicals and cell cycle inhibitors in driving them to become mature neuronal cells. Preliminary identification of neuronal differentiation was assessed by microscopic observation. RT-PCR, immunocytochemistry and western blotting were carried out to confirm and further characterise these differentiated cells.

Results: Neurogenic differentiation was assessed by RT-PCR, immunocytochemistry and western blotting. Differentiated flat BMSCs were shown to have an increasing expression of neuronal genes such as GAP43, Neurogenin 1 and Tau with respect to the exposure time to differentiation medium. Positive immunocytochemistry staining further confirm the identity of these differentiated cells.

Conclusion: This study shows that flat BMSCs can be differentiated into mature neurons. Flat BMSCs can potentially provide an abundant source of cells for autologous transplantation in treatment of neurological diseases.

1021/CPIP

Improving Clinical Practice of Intravenous Urography

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Aim: To improve the clinical practice of intravenous urography using turnaround time and report generation time as outcome measures.

Methods: A clinical practice improvement programme was implemented in January 2004. Historical data was used as a control. Measurements based on a radiology information system were used to track turn around time for patients and the generation of their reports. Multiple interventions based on brain-storming and multi-user input with interval measurements were used to reduce the measures.

Results: There is a reduction of the average turnaround time from 169 to 87 minutes while the 98% of reports could be generated within 24 hours after completion of the examination. This was achieved by using the collective wisdom of the process owners to build a foundation on which a standard protocol was based. Variation from the protocol was allowed after agreement among the staff involved.

Conclusion: It is possible, practical and profitable to improve clinical

practice using clinical practice programme methodology.

1022/CPIP

Reducing the Use and Duration of Physical Restraints in Disturbed Aggressive and Violent Psychiatric Patients

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Aim: The use of physical restraints is indispensable in Mental Health Institutions such as the Institute of Mental Health, Singapore (IMH). Oftentimes, they are crucial for staff and patient safety. However, restraints are unpleasant and sometimes dangerous. There have been reports of adverse clinical (e.g. DVT) and medicolegal complications. IMH sought to determine if the rates and durations of restraints could be reduced.

Methods: Nursing and medical staff were surveyed as to their beliefs regarding the reasons for over-restraining patients. Many thought that the existing protocols were too far-ranging and that restraints were used as first-line management of disturbed patients. New Restraint Protocols were drawn up and road shows conducted to convince staff of the rationale for the new procedures. Staff ($n = 25$ doctors and nurses) and patients ($n = 10$) were then surveyed as to their appreciation of and satisfaction with the new protocols.

Results: Post-implementation, the number and duration of restraints dropped by 74% and 37% respectively. The staff satisfaction survey revealed that most staff understood the need for the new protocol and found it easy to use. Staff generally realised patient safety was now enhanced. The patient satisfaction survey showed that 90% of patients supported the occasional use of restraints and applauded the hospital's initiative to reduce the number and rates of restraints.

Conclusion: It was learnt that reduction of restraints was possible, and patients and staff were happy with this. Future directions will look at other methods of managing violent patients.

1023/CPIP

Reducing Benzodiazepine Prescription at Point of Discharge

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Aim: Patients admitted to the acute wards in Woodbridge Hospital are frequently treated with oral benzodiazepines for management of their anxiety and insomnia but many of these are still being prescribed even at the point of discharge. To prevent dependency and other side effects, it is thus worthwhile to explore the unnecessary continuation of benzodiazepines at discharge.

Methods: The Pareto chart generated by the votes of the team revealed that the main reasons of continuing benzodiazepines at the point of discharge were: 1) medications not reviewed at the time of discharge, 2) patients' request for benzodiazepines and 3) treatment of agitation and insomnia. The interventions carried out included the use of reminders such as attaching reminder note on the computer, the inpatient medical record forms and casenotes. Suitable alternatives such as oral hydroxyzine were used whenever applicable.

Results: After 20 weeks of intervention, the prescribing of benzodiazepines at discharge reduced relatively by 16.28% and 49.89% from baseline in the female and male adult C class wards respectively. The combined results indicated a relative reduction of 30.23% from baseline (Student's t -test, $P = 0.00013$). In addition, the sustainability of not prescribing benzodiazepines to patients 10 weeks after discharge was improved relatively by 12.15%.

Conclusion: Benzodiazepines have an important and often indispensable therapeutic role for the short-term management of insomnia and anxiety symptoms. However, the risk of dependency can be minimised if one is mindful to review the need of continuing the medication at the point of discharge.

I024/CPIP**Reducing the Rate of Negative Appendicectomies – A Clinical Practice Improvement Project**

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Aim: Appendicectomy is a common emergency surgical procedure. Between January 2001 and June 2002, more than 900 appendicectomies were carried out in our institution. Analysis of our data showed a negative appendicectomy rate of 17.9% (9.3% males and 26.8% females). Appendicular perforation incidence was 6.6%. A Clinical Practice Improvement Programme (CPIP) project was initiated to reduce the negative appendicectomy rate.

Methods: A CPIP team was formed and looked into the potential factors influencing the relevant appendicectomy rates. A clinical protocol for the evaluation of patients with acute appendicitis was instituted by the general surgical department. Prospective data were collected on patients undergoing appendicectomies.

Results: For the study period September 2002 to December 2003 (excluding the SARS period), a total of 395 appendicectomies were carried out (61.2% males and 38.8% females). The overall negative appendicectomy rate was reduced from 17.9% to 11.6% (8.6% for males and 16.9% for females). The incidence of perforation was 7.3%.

Conclusion: Utilising the CPIP process, we were able to reduce our negative appendicectomy rates significantly. This resulted in reducing unnecessary appendicectomies, avoiding postoperative complications and savings in resources for both the patient and the institution

I025/CPIP**To Reduce the Waiting Time for Both Laboratory Testing and Treatment Procedures in the Subsidised Clinics by 30% from 15 minutes to 10 minutes Within 6 Months**HL EE¹, SC LEE², GT LIM³

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Aim: A survey on 178 patients was carried out to assess the satisfaction of patients waiting for the above test. The survey showed that all patients appreciated minimal waiting and half of the patients felt a reasonable waiting time for laboratory test was 5-10 minutes while the other half felt it was 11-15 minutes. The average waiting time for a laboratory test is 16.4 minutes. Prior to project implementation, all patients with laboratory investigations will be directed to T1/T2. The nursing staff will then inform the laboratory staff a procedure needs to be performed.

Methods: CPIP methodology (cause and effect brainstorming, pareto charts and a review of workflow processes) was employed. We concluded that a change in work processes is required. Patients requiring blood investigations and treatment will continue at T1/T2. The Laboratory for scraping purposes will be renamed T3 and patients requiring skin scraping will proceed to T3.

Results: Waiting times for laboratory procedures at T3 was improved from 16.4 minutes to 8.0 minutes (51.2% reduction) and patient dissatisfaction reduced from 73.4% to 0% (100.0% reduction). Investigations at T1/T2 on the other hand was improved to 2.7 minutes from 6.7 minutes (59.7% reduction) and the patients dissatisfaction at T1/T2 was 0% from 12.5% (100.0% reduction) This improvement translates to 0.5 and 0.25 fixed term equivalent for laboratory staff and nursing staff respectively.

Conclusion: Not only can we can fully meet patient's requirement and expectations with simple measures and at low cost but also translate it into cost savings for the centre.

I026/MPC**Severe Acute Respiratory Syndrome (SARS)-related Acute Respiratory Distress Syndrome (ARDS): Predicting the Clinical Outcome from Ventilatory Parameters**TK KWEK¹, TWK LEW¹, A EARNEST², Y CHAN¹, S LOO¹

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Aim: We noted in our cohort of 46 critically-ill SARS patients that some had

a short course of acute respiratory distress syndrome (ARDS) while others had a protracted course complicated by multi-organ dysfunction with high mortality. We hypothesised that this difference could be predicted earlier from ventilatory parameters (VP) measured at the bedside.

Methods: From 6 March to 6 June 2003, there were 39 ICU patients with probable SARS who required intubation and mechanical ventilation (MV). We employed a low-tidal-volume lung protective ventilation strategy, using volume or pressure control, in all patients. Based on the duration of MV, we divided patients into 2 groups (gp): Early Recovery (ER) gp if 14 days of MV were needed or if the patient died while on MV. The VP on D1, 4 and 7 were determined retrospectively from chart review and compared using the Mann Whitney test.

Results: VP were similar in both gps on D1 and 4 except for lower minute ventilation (mV) on D4 in the ER gp [9.0 vs 11.5, $P = 0.029$]. On D7, ER gp had significantly lower plateau pressures (PPr) [25.3 vs 32.0, $P = 0.025$], mV [8.9 vs 11.9, $P = 0.021$] and FiO₂ [0.5 vs 0.7, $P < 0.001$] and higher PaO₂/FiO₂ ratios [203.8 vs 114.7, $P = 0.008$] compared to the LR/M gp.

Conclusion: Patients with SARS related ARDS on MV who recover early tend to improve by D7 of MV and can be identified by improvements in their PPr, FiO₂, PaO₂/FiO₂ ratio and mV measured at the bedside.

I027/MPC**Aortic Valve Calcification Relates to Severity of Aortic Valve Stenosis But Not Coronary Artery Calcification**MY Y CHAN¹, KK POH¹, KY SETO², HH YANG³, LH LING³

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Aim: Aortic valve and coronary artery calcification are common in the elderly. Incidental aortic valve calcium (AVC) is often detected during computed tomography for coronary artery calcium (CAC) scores. We aim to determine the relationships between AVC, valvular stenosis and CAC.

Methods: Multislice detector computed tomography (MDCT) was performed in 57 patients (39 with significant stenosis, 12 sclerosis, 6 normal) to determine the extent of AVC and CAC. MDCT AVC was compared to echocardiographic indexes of aortic stenosis.

Results: Mean patient age was 68 ± 11 years with 60% males. The older cohort tended to have more calcification in the coronary arteries ($r = 0.41$, $P = 0.002$) and aortic valves ($r = 0.37$, $P = 0.005$). There was no significant difference in AVC and CAC between the genders ($Z = -0.9$, $P = 0.4$). AVC ranged from 0-7549 AU (medium = 515 AU) and CAC ranged from 0-8592 AU (medium = 303 AU). There was an inverse relation between the AVC and the degree of valvular opening (Spearman's $r = -0.75$, $P = 0.2$).

Conclusion: Both AVC and CAC increase with the age of patients. AVC on MSCT predicts severity of aortic stenosis. Heavy calcification suggests the presence of significant aortic stenosis that requires cardiological assessment. MDCT AVC was not significantly correlated to CAC and may not be useful as a marker for coronary artery disease in the elderly.

I028/MPC**Echocardiographic Predictors of Elevated N Pro in Patients with Aortic Valve Stenosis**MY Y CHAN¹, KK POH¹, HH YANG², LH LING²

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Aim: Natriuretic peptide (NT-proBNP) is elevated in symptomatic patients with aortic stenosis (AS) and may be a biochemical marker for AS severity. The relationships between NT-proBNP levels and the functional effects of AS on the LV myocardium in patients with normal LV ejection fraction (EF) have not however been adequately elucidated.

Methods: Transthoracic echocardiography was prospectively performed in 34 consecutive points with variable degrees of AS and EF $> 50\%$. Indexes of AS severity, systolic and diastolic function, and indexed left atrial and LV dimensions and LV mass were obtained. Spectral pulsed-wave tissue Doppler imaging (TDI) was performed at the septal and lateral corners of the mitral annulus. NT-proBNP plasma levels were measured independently.

Results: Mean patient age was 71 ± 12 years with 56% males. The mean

indexed aortic valve area (AVAI) was $0.63 \pm 0.23 \text{ cm}^2/\text{m}^2$. Severe (AVAI $0.75 \text{ cm}^2/\text{m}^2$) AS was present in 11, 10 and 13 points, respectively. NT-proBNP levels ranged from 23 to 35,000 ng/L (median 485 ng/L). Univariate predictors of elevated NT-proBNP were late diastolic mitral annular (A') velocities at both septal ($P = 0.02$) and lateral ($P = 0.009$) corners and indexed LV mass ($P = 0.04$) but not AVAI ($P = 0.24$). When conventional Doppler indexes of diastolic function, TDI diastolic velocities, indexed left atrial dimension and LV mass index were entered into a linear regression model, the sole independent predictor of NT-proBNP levels was lateral annular A' velocity ($\beta = 0.48$, $P < 0.01$)

Conclusion: Late diastolic annular velocities best-predicted (inversely) NT-proBNP levels in patients with AS and normal EF. This observation underscores the importance of atrial booster pump function in the pathophysiology of AS.

I029/MPC

Prevalence of Depression in Singapore – Results of the National Mental Health Survey of the Elderly 2003

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Aim: The proportion of the elderly ≥ 65 years in the population of Singapore is 7.6% and is increasing rapidly. The 3 main ethnic groups are the Chinese (77%), Malay (14%) and Indians (8%). This study aims to provide nationally representative estimates of the prevalence of depression in the elderly population of Singapore.

Methods: A cross-sectional community survey of a national ethnically-stratified random sample of older adults 60 years and above ($N = 1092$) was conducted. The Geriatric Mental State Examination (GMS) and the Automated Geriatric Examination for Computer Assisted Taxonomy (AGECAT) were used to diagnose depression.

Results: The overall population-weighted prevalence of depression for older adults ≥ 60 years, ≥ 65 years and ≥ 75 years were 3.1%, 3.5% and 3.9% respectively. The prevalence of depression in the age groups 60-64 years, 65-74 years, ≥ 75 years were 2.2%, 3.3% and 3.9% respectively (statistically insignificant). Among elderly ≥ 65 years old, the prevalence of depression in males and females was 3.2% and 3.8% respectively, (statistically insignificant); the prevalence of depression in Chinese, Malays and Indians were, 2.8%, 6.5% and 6.8% respectively. Gender-ethnic interaction was statistically significant ($P = 0.017$), being lowest in Chinese males (2.5%) and highest in Indian females (12.7%).

Conclusion: These nationally representative estimates of depression in the elderly population in Singapore identify gender and ethnic subgroups at high risk for further investigation and targeted intervention.

I030/MPC

Reversibility of Pulmonary Hypertension in Thyrotoxicosis (REPHIT Study)

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Aim: We aimed to test the hypothesis that thyrotoxicosis was a reversible cause of otherwise unexplained pulmonary hypertension.

Methods: All thyrotoxic patients with transthoracic echocardiograms (TTE) done before treatment were reviewed ($n = 115$). We found 20 patients with pulmonary artery systolic pressure (PASP) more than 40 mmHg (measured using tricuspid regurgitant maximum velocity plus estimated right atrial pressure), free thyroxine (FT4) more than 20 pmol/L and thyroid stimulating hormone (TSH) less than 0.05 mIU/L (20 patients). All 20 patients had a left ventricular ejection fraction (LVEF) more than 55% and there were no other significant echocardiographic abnormalities or pulmonary disease. 13/20 were recalled for a repeat TTE after they were rendered euthyroid. The difference in FT4 and PASP before and after treatment was calculated. P values were derived using the Wilcoxon Signed Ranks test.

Results: The median FT4 was 56.8 pmol/L at the 1st TTE and 13.9 pmol/L at the 2nd TTE with a median reduction in FT4 of 52 (range 8.2-88.2) pmol/

L, P value 0.005. There was a reduction in PASP from the 1st (median of 51 mmHg) to 2nd TTE (median of 36 mmHg) for all patients with a median reduction of 12 (range 1-33) mmHg, $P = 0.005$.

Conclusion: Thyrotoxicosis is a rare, reversible cause of pulmonary hypertension. A thyroid function test should thus be included in the workup of patients with otherwise unexplained pulmonary hypertension.

I031/MPC

Oral Sildenafil Therapy Improves Functional Class, 6-Minute Walk Test Distance and Physical Conditioning in Pulmonary Arterial Hypertension

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Aim: Treatment of pulmonary arterial hypertension (PAH) with continuous intravenous prostacyclin infusion is associated with high cost and complications related to delivery mechanisms. We aimed to determine if phosphodiesterase-5 inhibitor (sildenafil) may benefit PAH patients at reduced cost.

Methods: Fourteen patients (5 female, 9 male), aged 40 ± 17 years, with moderate to severe PAH, (5 primary pulmonary hypertension, 7 collagen vascular disease, 2 Eisenmenger syndrome) received oral sildenafil of mean daily dosage 154 ± 13 mg. Eleven (79%) patients were in WHO classes 3-4. These patients were followed up for 3 months with serial assessment of 6-minute walk test distance, WHO functional status, BORG dyspnoea score, pulmonary artery systolic pressures (PASP) via doppler echocardiography and Rand SF-36 Health Survey questionnaires.

Results: After 3 months of follow up, the WHO functional class improved from 3 ± 1 to 2 ± 1 ($P = 0.002$). Six-minute walk test distance improved from 275 ± 144 m to 387 ± 157 m ($P = 0.017$). BORG dyspnoea score also improved from 2.4 ± 2.5 to 0.6 ± 1.2 ($P = 0.1$). There was however no significant change in PASP, from 99.7 ± 24.0 mmHg to 93.6 ± 29.2 mmHg ($P = 0.1$). The "physical functioning" score in Rand SF-36 questionnaires showed significant improvement from 36 ± 10 to 70 ± 19 ($P = 0.004$).

Conclusion: Oral sildenafil therapy is effective in improving WHO functional class, exercise distance and physical conditioning scores in patients with PAH.

I032/MPC

Prediction of Endpoints in Patients with Renal Impairment by Nuclear and Echo Imaging Perined

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Aim: In patients with renal impairment. Left ventricular ejection fraction (LVEF) determined by echocardiography does not predict endpoints well because of variability in intravascular volume with diuretic use and dialysis. We hypothesise that the left atrial (LA) diameter may be a better predictor as the left atrial volume remains relatively stable despite fluctuations in intravascular volume.

Methods: We recruited 207 patients with serum creatinine above 150 mmol/L suspected to have coronary artery disease (CAD) without a prior history of myocardial infarction or stenosis $>50\%$ on prior coronary angiography. All patients had a resting echo-doppler and MPI scan performed. The LA diameter was measured by M-mode in the parasternal short-axis view and divided by the patient's body surface area to derive the LA diameter index. All patients were followed up for 24 months with the composite endpoint being all cause mortality and non-fatal myocardial infarction.

Results: Patients with an LA diameter index ≥ 22 (odds ratio 0.32; 95% CI, 0.28-0.41). Patients with a normal MPI scan also reached the endpoint less often (odds ratio 0.49; 95% CI, 0.29-0.67). Other significant predictors of the endpoint were age, presence of diabetes mellitus and LVEF.

Conclusion: In patients with renal impairment suspected to have CAD, a LA diameter index less than 22 cm/m^2 significantly predicts infarct-free survival at 24 months better than a normal MPI scan.

I033/MPC**Hemodynamic Significance of Mitral Stenosis: Utility of a Simple, Novel Index by 2-dimensional Echocardiography**SC SEOW¹, LP KOH¹, TC YEO¹¹Department of Cardiology, National University Hospital, Singapore

Aim: Assessment of mitral valve area by planimetry is accurate but operator dependent. It is technically challenging to determine the narrowest part of the "mitral funnel". Measuring the separation of the leaflet tips could be a simple and accurate surrogate measure of the mitral valve area.

Methods: We measured the distance between the tips of the mitral leaflets in mid-diastole in the parasternal long and apical 4 chamber views in 76 patients with mitral stenosis. The average of these 2 parameters was calculated to yield the MS Index. All measurements were obtained from the mean of 3 beats for patients in sinus rhythm and 5 beats for those with atrial fibrillation. The Index was then compared with 2 reference methods of mitral stenosis severity: mitral valve area by planimetry and pressure half-time.

Results: There was good correlation between the Index and mitral valve area by planimetry ($r = 0.907$, $P < 8$ mm and all patients with mild stenosis had a MS Index of >11 mm).

Conclusion: The MS Index is a simple, novel and reliable measure of mitral stenosis severity.

I034/MPC**Impact of Left Ventricular Diastolic Dysfunction on Left Atrial Remodelling and Function a Volumetric Analysis**SG TEO¹, H YANG², C PING¹, TC YEO¹¹Department of Cardiology, National University Hospital, Singapore,²Department of Cardiology, National University of Singapore, Singapore

Aim: Diastolic dysfunction may result in elevation of left ventricular and atrial pressures, resulting in left atrial (LA) remodelling. The aim of this study was to examine the effects of left ventricular diastolic dysfunction on LA remodelling and function.

Methods: We measured LA volume and function in 105 patients. Maximal (Volmax) and minimal LA volumes were measured using the ellipsoid method. LA Volmax was then indexed to the body surface area (BSA). The passive filling, conduit and active emptying volumes were estimated and corrected for BSA and LA Volmax.

Results: Indexed LA Volmax was strongly associated with diastolic function grade (Spearman $P < 0.0001$, $r_s = 0.701$). Compared to normal controls, corrected passive filling and conduit volumes were lower, and corrected active emptying volume was higher in patients with grade 1 diastolic dysfunction (0.38 vs 0.51, $P = 0.007$; 1.69 vs 3.29, $P < 0.0001$; 0.59 vs 0.44 $P = 0.001$), resulting in a similar corrected total emptying volume (0.97 vs 0.96, $P = ns$). Patients with higher grades of diastolic dysfunction, however, had lower corrected passive filling, conduit, active and total emptying volumes.

Conclusion: LA remodelling occurs in patients with diastolic dysfunction and LA volume expressed the severity of the diastolic dysfunction. Initially, the atrium compensates for changes in LV diastolic properties by augmenting active atrial contraction. As the severity of the diastolic dysfunction increases, this compensatory mechanism fails as atrial mechanical dysfunction sets in, resulting in lower total atrial emptying volume

I035/MPC**Novel Acoustic Analysis Predicts Severity of Aortic Valve Stenosis**MY CHAN¹, KK POH¹, ZY SUN², HH YANG³, CH CHEW², LH LING³¹Department of Cardiology, National University Hospital, Singapore, ²Dept of Mechanical Engineering, National University of Singapore, Singapore,³Department of Cardiology, National University of Singapore, Singapore

Aim: Phonocardiographic spectral ratios have been used to evaluate aortic stenosis (AS) severity. Using echocardiography as a non-invasive gold standard, we devised and assessed a new and more accurate wavelet-based acoustic method of quantifying AS severity.

Methods: ECG-gated acoustic data recorded with an electronic stethoscope with digital output were acquired from the praecordial aortic areas of 66 patients (22 in each group of severe AS, mild-to-moderate AS and sclerosis-

normal). These were subjected to energy based continuous wavelet transformation (CWT). A multi-peak detection computer algorithm was developed to extract the dominant frequency (DF) of the systolic murmur from CWT. Fast Fourier transformation was also performed to determine the spectral ratio of the high to low frequency range (Eh/EI). These parameters were compared to echocardiographic indexes of AS, determined independently.

Results: Mean patient age was 69 ± 11 years with 59% males. AVA ranged from 0.48 to 3.51 cm² (mean = 1.42 ± 0.75 cm²), DF from 30 to 350 Hz (medium = 100 Hz) and Eh/EI from 0.007 to 0.703 (medium = 0.455). The DF correlates with echocardiographic indexes were: aortic valve area ($r = -0.55$, $P \leq 0.001$), mean transvalvular gradient ($r = 0.53$, $P < 0.001$), aortic valve resistance ($r = 0.58$, $P < 0.001$), stroke work loss ($r = 0.60$, $P < 0.001$) and dimensionless index ($r = -0.55$, $P < 0.001$). The respective correlates for Eh/EI were: $r = -0.35$, $P = 0.010$ (valve area), $r = 0.37$, $P = 0.005$ (mean gradient), $r = 0.39$, $P = 0.006$ (valve resistance), $r = 0.42$, $P = 0.001$ (stroke work loss) and $r = -0.35$, $P = 0.008$ (dimensionless index).

Conclusion: Novel acoustic analyses hold promise for the evaluation of AS. Compared to spectral ratio, wavelet DF correlates better with AS severity. In technically difficult echocardiographic studies or cases of uncertain stenotic severity, this method of rapid non-invasive analysis provide additional value.

I036/MPC**Angiotensin Converting Enzyme Inhibitor and the Prevalence of Cough**TW WONG¹, XH HUANG¹, BT NG², W LIU¹¹Department of Cardiology, Tan Tock Seng Hospital, Singapore, ²Department of Pharmacy, Tan Tock Seng Hospital, Singapore

Aim: The most common side effect of angiotensin converting enzyme inhibitor (ACEI) therapy is cough. Cough affects compliance and is the most common reason for patient withdrawal from treatment. We aim to establish the prevalence and nature of ACEI-induced cough in Singapore.

Methods: This is a cross-sectional retrospective study of outpatient population in a tertiary hospital. Inclusion criteria were all patients who were taking ACEI or Angiotensin II receptor blocker (ARB) to replace ACEI cough.

Results: Six hundred and twenty-six patients were surveyed and 52 patients were excluded from our study. Of the remaining 574 patients, prevalence of cough was 32.8%. Mean age for patients who cough was higher; (66 SD:11) vs (63 SD:12) years. 41.6% of females taking ACEI had cough compared to 27.5% of male patients. There were no differences in underlying medical condition. Prevalence of cough was not dose related or ACEI specific. In the subgroup of 188 patients with cough, 56 (29.8%) patients reported severe cough, 129 (68.5%) patients coughed almost daily, 65 (34.6%) patients had nocturnal cough. Seventy-five (40%) patients had sleep disturbances from cough. Seventy-nine (42%) patients who cough were able to tolerate the cough. Eighty-seven (15.1% of 574) patients had to discontinue ACEI therapy.

Conclusion: This study indicates that cough is a common side effect of treatment with ACEI in Singapore population. Fifty-eight per cent of patients who cough required either cough remedies or discontinuation of ACEI. We recommend counselling all patients about the possible occurrence of cough before commencement of ACEI therapy.

I037/MPC**Scabies Outbreak in a HIV Unit**IH ESCUDERO¹, MIC CHEN¹, A EARNEST¹, YA LEO²¹Department of Clinical Epidemiology, Tan Tock Seng Hospital, Singapore,²Department of Infectious Disease, Tan Tock Seng Hospital, Singapore

Aim: To describe a protracted outbreak of scabies in a HIV unit, the role of informal living arrangements as a reservoir and measures instituted following recognition of outbreak.

Methods: Epidemiologic investigation consisting of a case-control study among HIV patients and staff of affected ward, outpatient clinic and Patient Care Centre to identify cases. AIDS status of all HIV patients was also noted. Empiric treatment with 1 dose of topical Malathion 0.5% was administered to all symptomatic HIV patients, those with exposure to the affected ward and PCC and all patients with positive skin scrape. Patients with persistently positive skin scrapings were given a second dose of Malathion 0.5% and

subsequent oral dose of Ivermectin.

Results: Nine cases of scabies were identified between May and October 2003. A second cluster of 4 cases was noted in January 2004 with 2 patients having a recurrence, a result of informal living arrangements. Risk factors for being infested with scabies among HIV patients include low CD4 count (OR: 1.29; 95% CI, 1.07-1.56) for every halving in values and not on anti-retroviral therapy (ART) (OR: 6.16; 95% CI, 1.06-35.90).

Conclusion: Protracted outbreaks of scabies can occur in HIV units. The difficulty in diagnosing Norwegian scabies and the mingling of inpatients with outpatients in both institutional and non-institutional support structures make for a challenging exercise in outbreak control. Scabitic infestations in HIV units should be treated with respect, and epidemiologic investigations and containment measures must look beyond institutional boundaries to find potential reservoirs.

1038/MPC

Database of Contact and Occupational Dermatoses Clinic in a Tertiary Referral Dermatological Centre

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Aim: The skin serves as an inter-phase between our bodies and the external environment. It protects us against environmental insults. In the course of our daily activities and work, we are constantly exposed to substances that can trigger a cutaneous reaction such as irritant contact dermatitis and allergic contact dermatitis. The aim of this poster is to present the prevalence and types of occupational and contact dermatitis seen in a tertiary dermatological centre and determine the most frequent triggers for these dermatitis.

Methods: This is a retrospective study. An on-line database collects epidemiological data of patients seen in occupational and contact dermatitis clinic in National Skin Centre over 22 months, from 21st March 2002 to 30th December 2003. It also records the results of the patch tests that were performed for the patients. (Test outcome was determined on the 3rd and 7th day post test).

Results: The most common dermatitis seen are allergic and irritant contact dermatitis. Nickel, dichromates and cobalt chlorides are among the common triggers.

Conclusion: Occupational and contact dermatitis results in man-hour loss, thus it has an economic impact. It is important to identify the common triggers so that preventive measures can be instituted into work place and patients can be educated so as to reduce incidence of such dermatitis.

1039/MPC

Adverse Skin Reactions to Personal Protective Equipment Against SARS

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Aim: Since the discovery of SARS, healthcare workers caring for patients in affected countries are exposed to regular use of personal protective equipment (PPE) such as the N95 mask, gloves and gowns, often using the equipment for hours at a time. Our aim is to determine the prevalence of adverse skin reactions to PPE amongst healthcare staff in Singapore hence determining whether PPE use poses a significant occupational health risk.

Methods: The target population was staff in National Skin Centre and the Departments of Emergency and Intensive Care in Tan Tock Seng Hospital. Questionnaires were used pertaining to the duration of use of PPE and adverse skin reactions arising from their use.

Results: Of those asked to participate, 322 (94.7%) agreed. 14.3% of respondents were doctors, 73.0% nurses, and 12.7% comprised other ancillary staff. Ages ranged from 20 to 63 years, the majority were female (85.7%) and Chinese (53.7%). 109 (35.5%) of the 307 who used masks regularly reported acne (59.6%), facial itch (51.4%) and rash (35.8%) from N95 use. 64 (21.4%) of the 299 who used gloves regularly reported dry skin (73.4%), itch (56.3%) and rash (37.5%). Only 4 (1.6%) of the 258 who wore gowns regularly reported itch (100%) and rash (75%).

Conclusion: The use of PPE, in particular, the N95 mask, is associated with high rates of adverse skin reactions. There is a need to find suitable

alternatives for affected staff and to encourage awareness among staff of the role of dermatologists in their care.

1040/MPC

Spectrum of Skin Disorders Among HIV Patients in Singapore and its Relationship to the Degree of Immunosuppression

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Aim: Skin disorders are extremely common in and of significant morbidity to HIV-infected individuals. However, data on its prevalence and association with CD4 count among Asians are lacking.

Methods: This cross-sectional study examined skin disorders among HIV-positive outpatients in Communicable Disease Centre between August 2003 and February 2004. Information collected included current and past skin problems, corresponding CD4 count, antiretroviral therapy and HIV transmission modes. The association between disease prevalence with CD4 count was evaluated using univariate and multivariate analyses.

Results: Ninety-six patients (Male:Female = 8:1) were enrolled. The most common mode of HIV transmission was heterosexual (75%) followed by homosexual/bisexual contacts (22%) and intravenous drug abuse (3%). The distribution of patients in terms of current CD4 cell counts was 38.5%.

Conclusion: The high prevalence of psoriasis, PPE and adverse drug eruptions relative to cutaneous infections, together with the absence of skin tumours, distinguish this report from western studies. Low CD4 cell count is significantly associated with psoriasis, PPE and adverse drug eruptions.

1041/MPC

Cardiovascular Risk in Patients with First Episode Psychosis

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Aim: Patients with schizophrenia, schizoaffective disorder, and affective psychosis are at increased risk of having risk factors for cardiovascular diseases. **Aim:** To describe the cardiovascular risk profile of drug-naïve patients diagnosed with schizophrenia, and assesses the risk of coronary heart disease based on the Framingham Heart Study.

Methods: Subjects are recruited from the Early Psychosis Intervention Programme. Control data were obtained from a representative cohort of the general population. Risk factors were obtained based on history and relevant laboratory investigations.

Results: Our patients were at higher risk of coronary heart disease, based on their risk scores as tabulated by the Framingham Heart Study. From our study results, patients were twice as likely as the control group to develop coronary heart disease ($P < 0.001$). The major contributing factor to patients' increased risk was the higher prevalence of smoking in patients (32.29%) compared to the control group (7.65%).

Conclusion: Interventions directed at smoking cessation should be targeted at patients with first episode psychosis.

1043/MPC

Who Dies Within 24 Hours of Admission from the Emergency Department?

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Aim: To describe the characteristics of patients who died within 24 hours of admission from the Emergency Department (ED).

Methods: Computer records from the Tan Tock Seng Hospital Office of Quality Management and the ED were obtained for all patients admitted by ED from 1 September 2003 to 31 January 2004. Patients who died within 24 hours of admission from ED were identified and retrospective chart reviews were done.

Results: The ED admitted 18,296 patients during the study period of which

145 (0.8%) died within 24 hours of admission. There were 84.8% Chinese, 6.2% Indian, 6.2% Malay and 2.1% other ethnic groups. The mean age of 69 years (Standard deviation [SD] 15.3) of the 85 (58.6%) men was significantly younger ($P = 0.001$) than 76.8 years (SD 12.4) of the 60 (41.4%) women. ED diagnoses were classified as infective (31.7%), cardiac (20.7%), neurological (13.8%), malignancy (9.7%), surgical and multiple trauma (9.6%), endocrine (4.4%) and others (8.3%). The commonest diagnoses were pneumonia (18.4%), coronary events (12.4%) and sepsis (11.3%).

Conclusion: Most of the 24-hour deaths occurred in the elderly with infective and cardiac causes accounting for over 50% of the deaths. Further studies are required to assess the ED management of these patients.

1044/MPC

A Profile of Male Victims of Domestic Violence Presenting to an Emergency Department in Singapore

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Aim: Male victims of domestic violence have received considerably less attention than female victims. The characteristics of male victims are poorly understood. We attempt to profile male victims of intimate partner violence, and investigate the differences in the characteristics of male and female victims presenting to an emergency department in Singapore.

Methods: 149 patients, presenting with injuries inflicted by their partner, were surveyed. Their demographics, education levels, assault characteristics and knowledge of help services were analyzed and compared.

Results: 9.4% of the victims were male. The proportion of elderly (42.9% vs 5.9%; $P < 0.0001$) and tertiary educated (35.7% vs 10.4%; $P = 0.022$) victims was significantly higher amongst male compared to female victims. Males were more likely to be face weapon-assaults (50.0% vs 10.4%; $P < 0.0001$) and suffer open wounds (50.0% vs 10.4%; $P < 0.0001$) than females. Majority of males confess to previous abuse (78.6%), while female victims were more likely to report their first abuse (77.0%; $P < 0.0001$). Males and females were equally aware of social and legal services.

Conclusion: Male domestic violence is a significant issue and deserves greater attention. Male victims of intimate partner are enduring years of abuse before coming forward. The severity of injury at the time of presentation is greater. Furthermore, our results suggest that male domestic violence is more common amongst well-educated couples.

1045/MPC

Addiction Inpatient Rehab Programme – Improving Attendance at Psychoeducation Sessions

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Aim: To increase attendance rate at our psycho-educational sessions from a baseline 58% to above 70%. Our inpatient addiction ward at Institute of Mental Health caters to multiracial and multilingual addicts who have chronic alcohol/substance dependence with multiple substance related and social problems requiring inpatient detox and rehab services.

Methods: Using the Plan Do Study Act (PDSA) method, we focused on various strategies after brainstorming our cause and effect diagram. We identified 4 areas delineated by our pareto chart, namely poor insight, feeling sick excuses, no programme variety and limited medium of instruction for immediate improvements.

Results: At 15 weeks' post-intervention, we found a mean average attendance of 74.3%, significantly above our baseline rate of 57.8%. When adjusted to exclude the low holiday segment occupancy period (Chinese New Year), the attendance rate was 77.2% compared to baseline. Our patient satisfaction score was found to be 81.2%.

Conclusion: A range of specific strategies targeted at the main causal factors could be combined for optimal outcomes in both attendance rates and patient satisfaction. Such strategies include a combination of strategies involving motivational enhancement, social problem assistance, physical examination, staff monitoring, creative involvement and interactions with ongoing multilingual translations and interpretations.

1046/MPC

Risk Factors for Depressive Disorders in Singapore Adults

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Aim: This epidemiological study set out to determine the risk factors for Depressive Disorders in the adult population of Singapore.

Methods: A stratified random sample of 2847 adults aged 20 to 59 years in the general population was assessed for the presence of Depressive Disorders. The diagnostic instrument used was Schedule for Clinical Assessment in Neuropsychiatry (SCAN). Social, economic and demographic details were recorded.

Results: The 161 individuals diagnosed to have Depressive Disorders (Major Depressive Disorder or Dysthymia) were compared to 2868 without the diagnosis. Univariate and multivariate analyses revealed that monthly income <\$3000 ($P = 0.000$), being unemployed, part-time employed or retired ($p = 0.001$) were significant economic risk factors associated with Depressive Disorder. Being widowed ($P = 0.001$), divorced/separated ($P = 0.001$), having never married ($P = 0.001$), and living alone ($P = 0.001$) were significant social risk factors. The presence of one or more medical problems ($P = 0.001$), a family history of mental illness ($P = 0.001$) and the occurrence of one or more threatening experiences ($P = 0.001$) were the other significant risk factors identified. After accounting for these factors, females and Chinese and Indians remained more likely to have Depressive Disorder.

Conclusion: Understanding the risk factors that predispose individuals to depression is essential in the management of the disorder. Awareness of the risk factors and the use of suitable screening measures by primary care providers for at-risk individuals may result in early detection, effective interventions, and reduced disability in individuals suffering from Depressive Disorders.

1047/MPC

The Prevalence of Psychiatric Disorders in Singapore Adults

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Aim: This landmark epidemiological study set out to determine the prevalence of recent psychiatric disorders in the adult general population.

Methods: A random sample, stratified by ethnicity, of adults aged 20 to 59 years living in the community were screened in 2003 for the presence of mental health disorder with the General Health Questionnaire (GHQ-12), and those scoring ≥ 2 were administered the Schedule for Clinical Assessment in Neuropsychiatry (SCAN) which generated the diagnoses of Anxiety and Depressive Disorders. Of 3875 individuals contacted, 2847 participated in the study (response rate: 75.2%).

Results: A total of 228 subjects were identified as having psychiatric disorders, including 40 cases which were not identified by SCAN as cases but were on psychiatric treatment. Only a total of 62 cases (27%) were on psychiatric treatment. Among 188 SCAN detected cases, only 36 (19%) reported having a mental health problem. The population-weighted recent prevalence of psychiatric disorders (SCAN and Non-SCAN cases) was 8.2% (95% CI 6.6-9.8). The prevalence of Depressive Disorders (Major Depressive Disorder and Dysthymia) was 4.9% (95% CI 3.7-6.2); female: 5.5% (95% CI 4.0-7.1), male: 4.3% (95% CI 2.4-6.3). The prevalence of Depressive Disorders was lowest in Malay males: 2.5% (95% CI 0.7-4.2), and highest in Indian females: 7.7% (95% CI 5.5-9.9).

Conclusion: This study shows that psychiatric disorders significantly affect the adult population of Singapore. These important findings should drive effective allocation of resources for case management. Early detection of these disorders in sub-populations at higher risk should result in improved treatment outcomes and reduced disability.

I048/MPC**Evaluation of Osteoporosis Treatment After a Fragility Hip Fracture**R RAMASON¹, WS LIM¹, YY SITOH¹, NH ISMAIL¹, HP WONG²¹Department of Geriatric Medicine, Tan Tock Seng Hospital, Singapore,²Department of Orthopaedic Surgery, Tan Tock Seng Hospital, Singapore

Aim: Hip fractures are a serious consequence to osteoporosis and are associated with substantial morbidity and mortality. The primary objective of this study was to assess if osteoporosis treatment was initiated in patients admitted with osteoporotic hip fracture. Secondary objectives included the evaluation of the rate of institutionalisation in the elderly post hip fracture.

Methods: A retrospective chart review was carried out on 216 consecutive patients admitted to Tan Tock Seng Hospital with a new low-energy hip fracture from January to December 1999. Osteoporosis treatment included supplemental calcium (alone or with vitD) or antiresorptive therapy (bisphosphonates, SERMs). A retrospective chart review was done to show the place of discharge from acute hospital or community hospital.

Results: A total of 216 case notes of patients with hip fractures were analysed. 164 (76%) were women; mean $\pm$ SD age at time of fracture was 80.7 ± 9.1 years and 52 (24%) were men with mean age of 76.3 ± 10.1 years. 63% were given treatment for their osteoporosis, 31% did not receive treatment for osteoporosis, 6% died before discharge. Furthermore, 21% had nursing homes as their final discharge destination.

Conclusion: The osteoporosis treatment rate in our cohort compared very favourably with results of other studies conducted around a similar period (typically 4%-29%) treatment rate on discharge. Hip fracture is a risk factor for institutionalisation with 11% community dwellers eventually going to nursing homes post discharge.

I049/MPC**Risk Factors Associated with Depression in Older Adults in Singapore**PC CHIAM¹, TP NG², A ANG¹, PS ONG¹, LL TAN¹, EH KUA²

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Aim: To examine risk factors associated with depression in older adults.

Methods: We analysed data of subjects with depression ($n = 48$) and without depression ($n = 1044$) from a cross-sectional study of a national ethnically-stratified random sample of 1092 older adults aged 60 years and above living in the community. Depression was diagnosed using the Geriatric Mental State Examination (GMS) and Automated Geriatric Examination for Computer Assisted Taxonomy (AGECAT) was used to diagnose depression. Risk factors examined included social, economic and medical data.

Results: Significant independent risk factors identified from weighted multivariate logistic regression analyses were Malay ethnicity (OR = 2.9 vs Chinese), being self-employed (OR = 6.8 vs retired/housewives), living in 1-2 room public housing (OR = 8.0 vs living in 5 room public housing or private housing), higher post-secondary educational level (OR = 4.7 vs no formal education), and having 3 or more medical comorbidities (OR = 18.4 vs having no medical comorbidities). Depression was not associated with gender, marital status or religion.

Conclusion: Important socio-economic and psychosocial factors and having medical conditions are associated with depression in older adults in Singapore. These information used in directing policy and planning of elderly health and social services may reduce morbidity and burdens and cost of care.

I050/MPC**Prognostic Factors in Patients with Acute Myeloid Leukaemias and High-Grade Myelodysplastic Syndromes—A Single Institution Evaluation**LG LAU¹, WJ CHNG¹, MY NORAINOON¹, KH ONG², LK TAN³, BMF MOW¹, YK KUEH⁴

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Aim: To identify and evaluate prognostic factors that influence overall (OS) and disease-free survival (DFS) in patients with myeloid malignancies.

Methods: A retrospective clinical and histo-cytogenetic-molecular data review of all newly-diagnosed patients with myeloid malignancies at the National University Hospital from 2000 to 2003.

Results: A total of 93 patients were identified (median age: 55 years). The breakdown of the subtypes of myeloid malignancies is: RAEB 11.8%, RAEB-T 4.3%, M0 3.2%, M1 8.6%, M2 24.7%, M3 10.8%, M4 16.1%, M5 9.7%, M6 6.5%, M7 2.2% and biphenotypic 2.2%. With median follow-up of 6 months (range 0.1-49.3), 16.1% were lost to follow-up, 59.1% had died and 24.7% were alive. The median OS and DFS were 5.2 months and 9.3 months respectively. In univariate analysis, age, presenting white cell count (TWC), FAB M3 morphology, LDH levels, cytogenetics, and response to first induction chemotherapy were identified as prognostic factors for both DFS and OS. In addition, response to induction and stem cell transplantation (SCT) were also prognostic factors for OS. In multivariate analysis, presenting TWC, LDH levels, antecedent haematologic disease, response to induction and SCT were significant prognostic factors for OS while presenting TWC, cytogenetics and FLT3 mutation were significant for DFS.

Conclusion: Prognostic factors for a cohort of local patients with myeloid malignancies were identified in this retrospective study. These should be validated in on-going analysis and, eventually, incorporated into management planning algorithm for patients with newly-diagnosed myeloid malignancies.

I051/MPC**Novel CYP2C9 Genetic Variants in Asians and Their Influence on Maintenance Warfarin Dose**SC RANKIN¹, F ZHAO¹, C LOKE², HB KHOR¹, JY GUO¹, HS LEE³, TS WU², T TAN⁴, TC LIU¹, YT LIM⁵, YM WONG⁶, A WONG⁷, KS NG⁸, BC GOH¹, SC LEE¹

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Aim: Commonly occurring genetic variants in CYP2C9 are associated with enhanced patient sensitivity to warfarin. Inter-ethnic differences in warfarin dose requirement have been described in Asians, and we postulate that this could be related to genetic variants of CYP2C9 that are unique to ethnic groups.

Methods: We prospectively genotyped patients who were receiving a stable daily warfarin dose to maintain International Normalised Ratio (INR) between 2-3 through comprehensive sequencing of the promoter and coding regions of CYP2C9.

Results: The mean weight-adjusted warfarin maintenance dose was significantly lower for Malays and Chinese than Indians (pC, 374G>A, 485C>A, 895A>G, 1144C>T, 1190A>C and 1362G>C). CYP2C9*3 but not CYP2C9*2 was found in Chinese and Malay patients, and carriers of the CYP2C9*3 variant in Chinese ($P < 0.01$) and Indians ($P < 0.01$) but not Malays ($P = 0.77$) required less warfarin. The influence of the novel exonic variants on warfarin dose requirement was unclear as they were rare, but the lower warfarin dose requirement for Chinese and Malays existed despite omitting individuals with any coding region variants from analysis. This study is ongoing, with over 200 patients enrolled and 125 patients genotyped. More data will be available at the congress.

Conclusion: Inter-ethnic differences in warfarin dosing in Asians may be due to other genetic, dietary or environmental influences; however, these novel genetic variants warrant further characterisation through functional studies.

I052/MPC**Clinical Genetic Counselling and Genetic Testing for Hereditary Breast Cancer in Singapore**WS CHIENG¹, SC LEE¹, P IAU², R YIP³

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Aim: A Cancer Genetics Clinic that manages hereditary cancers has been established in the National University Hospital since 2001.

Methods: We report the clinical profile of women with breast cancer evaluated in this clinic.

Results: 114/128 (89%) patients evaluated were high-risk with at least 10% chance of carrying a BRCA1/2 mutation, and were offered genetic counselling and testing. The majority were Chinese (73%), median age was 38 (range 25-83) years. 78% were married, 50% had children. 63/114 (55%) had young breast cancer, below age 40, with no family history of breast or ovarian cancer. 42/114 (37%) had at least 1 first- or second-degree relative with breast cancer. 9/114 (8%) had family history of breast and ovarian cancer. 20/114 (18%) undertook genetic testing, of which 8/20 (40%) and 3/20 (15%) had a deleterious mutation or missense mutation of uncertain significance respectively. Predictive testing for cancer-free family members is underway to identify high-risk subjects for screening. Cost was a major barrier to testing: only 4/82 (5%) women were tested at S\$4500 prior to availability of government subsidy; the majority tested after September 2003, with a government grant that provided 100% subsidy to women with at least 30% probability of carrying a mutation, whereupon 16/32 (50%) eligible patients undertook testing.

Conclusion: BRCA1/2 families in Singapore may be identified through genetic testing. Governmental efforts to overcome cost barriers are instrumental to optimise use of this specialised service to identify high-risk subjects for screening to reduce cancer burden.

I053/MPC

Clinical Spectrum of Severe Acute Respiratory Syndrome in Exposed Healthcare Workers

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Aim: The nosocomial outbreak of severe acute respiratory syndrome (SARS) in Singapore began in early March 2003 at Tan Tock Seng Hospital (TTSH), at a time when SARS was not recognised and no infection control measures were in place. We assessed the clinical spectrum of SARS coronavirus infection in exposed healthcare workers (HCW).

Methods: We did a seroepidemiological study amongst all HCW exposed to the first 3 SARS cases at TTSH in the first month of the outbreak. HCW completed a detailed questionnaire on duration and type of exposure to SARS patients and provided blood samples. ELISA for SARS coronavirus, and quantitative antibody titres were performed, with the laboratory personnel blinded to clinical data.

Results: Of 99 exposed HCW, 81 consented to participate. 45 (55.6%) cases were SARS serology positive. Of the 45 SARS positive cases, 38 (84%) had pneumonic changes, 3 (6%) had fever and respiratory symptoms but no pneumonia, 2 (4%) were totally asymptomatic and another 2 (4%) had no respiratory symptoms or fever, but had diarrhoea. The titres for SARS antibodies was significantly higher in probable SARS cases versus asymptomatic cases. On univariate analysis, there was no difference in the duration and type of exposure to the source cases between pneumonic SARS cases and asymptomatic SARS serology positive cases.

Conclusion: The presence of asymptomatic SARS cases concurs with findings from Hong Kong, and may explain cases of SARS-CoV pneumonia who had no obvious contact with other patients with SARS.

I054/MPC

MRSA and SARS

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Aim: To examine the effect on MRSA rates of workflow changes, isolation policies and infection control precautions during SARS.

Methods: Known MRSA patients were taken out of isolation rooms and placed in general wards as priority for isolation rooms was given to patients

who required airborne precautions. Work practices were changed: patients were examined by fewer doctors during rounds, and case notes kept away from patients' bed-side. Compliance to hand hygiene was excellent because of fear of getting SARS.

Results: Though the total number of MRSA cases fell during the second quarter when there were SARS patients in TTSH, the rate per 1000 patient-days was paradoxically increased because of a reduction in the denominator. The trend was similar when the denominator was 1000 deaths/discharges. The increase corresponded to an increase in the length of stay. Both rates returned to pre-SARS levels after the hospital resumed normal admissions and the average length of stay returned to baseline.

Conclusion: Prolonged length of stay plus placing of MRSA patients in general wards contributed to increased MRSA rates despite heightened infection control awareness during SARS.

I055/MPC

Factors Affecting Outcome in Diabetic Peritoneal Dialysis (PD) Patients

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Aim: Diabetic end-stage renal failure (ESRF) patients generally have a poorer outcome than those without diabetes mellitus (DM). Our study was aimed at defining factors that was associated with poor outcome in the diabetic PD population.

Methods: This retrospective study included all patients who started PD in 2002 to March 2003. All analyses were done comparing diabetic PD patients with those that did not have DM. We chose 4 parameters (age, serum albumin, haemoglobin and C reactive protein) that have been reported in other studies to affect survival.

Results: A total of 53 diabetic and 25 non-diabetic PD patients were included in the study. The mean follow-up time was 17.2 ± 6.8 months. The mean ($\pm$ SD) survival for the diabetic PD patients was 16.9 ± 7.1 months as compared to the 17.7 ± 6.4 months for those without DM. Diabetic PD patients had lower serum albumin ($P = 0.019$) and higher C reactive protein ($P = 0.014$) compared to the non-diabetic PD patients. The diabetic PD patients were not older ($P = 0.258$) and did not have significantly different haemoglobin ($P = 0.897$). A higher C reactive protein was also significantly associated with lower serum albumin in the diabetic as well as the overall population. A higher C reactive protein and lower serum albumin were both independent predictors of survival in diabetic PD patients.

Conclusion: Diabetic PD patients have lower serum albumin and higher C reactive protein. It may be the combination of these 2 factors (inflammation-malnutrition syndrome) that adversely affects the outcome of the diabetic PD population.

I056/MPC

Survival on Peritoneal Dialysis is Adversely Associated with Serum Albumin

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Aim: Peritoneal dialysis (PD) is commonly practiced in Singapore. As in haemodialysis, survival on PD can be influenced by many factors including nutritional status. The aim of our study was to correlate survival on PD with serum albumin (as a surrogate marker for nutrition) at incident of dialysis.

Methods: This retrospective study included all patients who started PD in 2002 to March 2003. Incident biochemical markers were defined as the parameters that were taken at 3 months after starting PD. Survival on PD was analysed along 2 distinct groups of serum albumin. Group I had serum albumin <33 g/L and Group II had serum albumin ≥ 33 g/L.

Results: A total of 78 patients were included (53.8% females). The mean age ($\pm$ SD) was 64.1 ± 12.6 years, with 67.9% diabetics. Lower serum albumin was associated with lower haemoglobin ($P = 0.012$), and higher C reactive protein (CRP) ($P = 0.024$). It was not associated with patients' age. There were 40 patients (51%) in Group I and 38 patients (48%) in Group II. Serum albumin at incident predicted survival on this PD population ($P = 0.0023$). This association was not changed when adjusted for haemoglobin, CRP, or age.

Conclusion: Serum albumin at incident is a predictor of poor outcome on PD.

This supports the need to have intensive nutritional intervention even in the pre-end stage renal failure stage.

1057/MPC

Impact of Glycaemic Control on Glomerular Filtration Rate (GFR) Changes Across Different Stages of Chronic Kidney Disease (CKD) in Patients with Diabetic Nephropathy

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Aim: To determine whether reduction in HbA1c will retard the progression of diabetic nephropathy in poorly glycaemic controlled patients at different stages of CKD classified by National Kidney Foundation K/DOQI guidelines.

Methods: Data from 51 diabetic nephropathy patients with HbA1c $\geq 8\%$ were analysed retrospectively from September 2003 to April 2004. Thirty-five patients with significant reduction in HbA1c were selected. GFR at baseline and at the end of follow-up were calculated by MDRD formula. The patients were stratified into 5 stages of CKD. The stages were GFR >90 ($n = 2$), 60-89 ($n = 9$), 30-59 ($n = 14$), 15-29 ($n = 9$) and <15 ($n = 1$) ml/min/1.73m². One patient in stage 5 was excluded.

Results: Mean age of patients in stage 1 to 4 of CKD was 47.5 ± 2.1 , 56.4 ± 7.7 , 61.2 ± 8.1 and 63.6 ± 4.0 years. The means of HbA1c reduction were 1.9, 2.0, 1.7 and 2.5 respectively. Average reduction in GFR in stage 2 and stage 3 were 15.5 ($P = 0.03$) and 6.4 ($P = 0.001$) ml/min/1.73m². The rates of GFR reduction were 22% ($P = 0.033$) and 17% ($P = 0.000$). However, patients in stage 4 did not seem to have significant decrease in GFR that is probably due to lower baseline nephron mass.

Conclusion: The rates of decline in GFR were significant in patients with poor glycaemic control. Interestingly, the rates of decline were faster in patients with stage 2 and 3 CKD despite improvement in glycaemic control. Further studies with larger sample size and other related factors are warranted.

1058/MPC

Impact of Intensive Multi-disciplinary Approach on Reduction of HbA1c in Diabetic Nephropathy Patients

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Aim: To evaluate the impact of intensive multi-disciplinary approach on reduction of HbA1c in diabetic nephropathy patients with poor glycaemic control.

Methods: Fifty-one patients (49% male; 67% Chinese, mean age 60 ± 8.25 years) with diabetic nephropathy and HbA1c $\geq 8\%$ were studied prospectively from 1 September 2003 to 31 March 2004. All patients were followed up intensively by physicians (between 2-5 visits) to have medications (oral hypoglycaemic agents and/or insulin) adjusted, compliance evaluated and diabetic-related complications assessed. Patients were seen by dietitians at least 2 visits for dietary counselling and lifestyle modification. Social and financial issues were assessed and followed up by medical social workers. The patients' progress was monitored by registered nurses. Blood pressure, HbA1c, renal function and proteinuria were measured periodically.

Results: Thirty-five patients (68.6%) had significant reduction in HbA1c after 6 months of intensive multi-disciplinary approach. HbA1c was unchanged in 2 patients. Mean HbA1c at baseline and post intervention in patients with improved glycaemic control were 10.5% and 8.6% respectively ($P \leq 7\%$). Among 51 patients, the baseline and latest mean HbA1c were 10.4% and 9.8% respectively ($P > 0.05$). There were no differences in gender, ethnicity and anti-diabetic medications used in 51 patients.

Conclusion: Intensive multi-disciplinary approach had significant impact on reduction of HbA1c in diabetic nephropathy patients with poor glycaemic control. To achieve and maintain optimal HbA1c ($\leq 7\%$) is challenging. Multi-level task and co-operation among healthcare professionals and patients must be enforced.

1059/MPC

Effect of Handphone Use on the Mechanical Ventilator in ICU

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Aim: A recent report highlighted that a handphone may interfere with the performance of the mechanical ventilator (MV), especially if it does not have proper electromagnetic shielding. As the handphone tested is not used in Singapore, we decided to test common local handphones on the 1800 Hz frequency network.

Methods: The PB840 (Tyco) MV was set up at an empty ICU cubicle ventilating a test lung. Set distances (0 cm, 30 cm, 50 cm, and 100 cm) from the ventilator were marked and a height of 100 cm was used to place the handphones tested. The handphones were allowed to ring for 5 rings and then the call was accepted. A total of 7 handphones were used (5 different Nokia models: 6610, 6600, 8310, 6230, 3100; 1 Samsung S500; 1 Panasonic 6D88).

Results: When the handphones rang, the changes seen were increases in respiratory rate, and increases in tidal volumes. These were seen with all handphones at 0 cm from the ventilator. The Nokia 6600 and Samsung S500 did not affect the ventilator unless they were placed at 30 cm or less from the ventilator. Only 2 handphones caused changes at 100 cm (Nokia 6230 and the Panasonic 6D88). There were no shutdowns.

Conclusion: Handphones that are ringing may influence the performance of the PB840 MV if placed less than 100 cm from the ventilator. Adequate electromagnetic shielding for these devices should be considered if handphones are to be used in the ICU setting.

1060/MPC

Angiotensin Receptor Antagonist (ARB) Versus Angiotensin Converting Enzyme (ACE) Inhibitor in Asian Subjects with Type 2 Diabetes and Albuminuria – A Randomised Cross Over Study

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Aim: Subjects with type 2 diabetes mellitus (T2DM) and albuminuria are at risk for progressive diabetic nephropathy (DN). No randomised controlled trial has been performed to directly compare the antiproteinuric efficacy of ARB vs ACE inhibitor

Methods: Forty ARB- and ACEI-naïve T2DM subjects with albuminuria (>30 mg/g creatinine) were given either 50 mg of Losartan (L) (ARB) or 20 mg of Quinapril (Q) (ACE inhibitor) (50% maximum dose) for 4 weeks with 4 weeks wash-out period in-between interventions in a cross over fashion. The order of intervention was randomised. The primary endpoint was reduction of albuminuria

Results: Among the 40 subjects, 62% were Chinese, 33% Malays and 5% Indians; 67% were male. The mean age (SD) was 54(10) years, BMI 27.1(4.6) kgm⁻², waist 92(13) cm, baseline systolic BP (SBP) 135(15) mmHg, diastolic BP (DBP) 83(9) mmHg, FPG 9.3(2.7) mM, HbA1c 8.3(1.8)%, serum potassium 4.2(0.4) mM, serum creatinine 76.4(17.9) μ M, urinary albumin/creatinine ratio (ACR) 445 (978) mg/g. Blood pressure reduction on both interventions was similar [SBP: L 3(14) vs Q 2(12) mmHg, $P = 0.65$; DBP: L 1(9) vs Q 2(8) mmHg, $P = 0.66$]. However, amelioration of albuminuria was significantly greater with Losartan [L vs Q: -367 (973) vs -21 (318) mg/g, $P = 0.01$].

Conclusion: In Asian subjects with T2DM and albuminuria, 50 mg of Losartan appears to have greater antiproteinuric effect than 20 mg of Quinapril in spite of comparable reduction in blood pressure.

1061/MPC

The Relationship Between NCEP Defined Metabolic Syndrome and Albuminuria in Asians

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Aim: Little is known on whether Asian subjects with NCEP defined metabolic syndrome are also associated with albuminuria.

Methods: We studied the relationship between NCEP-defined metabolic syndrome and albuminuria in 616 Asian healthcare workers. Elevated waist circumference was defined as male ≥ 90 cm and female ≥ 80 cm. Albuminuria was defined as spot urinary albumin over creatinine ratio ≥ 30 mcg/mg. A factor of 0.68 was used to adjust for gender differences in urinary creatinine excretion.

Results: 58% were Chinese (C), 25% Malays (M) and 17% Indians (I); 71% were females. The mean age ($\pm$ SD) was 38 ± 12 years, BMI 24.1 ± 4.6 kgm⁻², waist 73 ± 17 cm, systolic BP (SBP) 119 ± 15 mm Hg, diastolic BP (DBP) 76 ± 10 mm Hg, FPG 5.3 ± 1.4 mM. Median urinary albumin/creatinine ratio (ACR) was 8.8 mcg/mg (95% CI 5-96). Only 6.7% of the participants met the NCEP criteria for the diagnosis of metabolic syndrome. Subjects with the metabolic syndrome are older (43 ± 12 vs 37 ± 11 years; $P < 0.001$) and more likely to be of Indian ethnicity (I vs M & C: 12.4% vs 6.6% & 5.0%; $P = 0.03$). The proportion of albuminuric subjects among individuals with and without the metabolic syndrome differed significantly (30 vs. 12% respectively, $P = 0.005$). Using logistic regression with adjustment for differences in age, presence of the metabolic syndrome was associated with an increased likelihood of albuminuria (odds ratio 1.57, 95% CI 1.23-2.69; $P = 0.048$).

Conclusion: Asian subjects with NCEP defined metabolic syndrome are also at increased risk for albuminuria.

I062/MPC

The Non-hydrotic "Bart's Hydrops" (homozygous alpha-0 thalassaemia)

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Aim: Most fetuses with homozygous alpha-0 thalassaemia are diagnosed in-utero and the pregnancies terminated. A non-hydrotic presentation in pregnancy and newborn period is a rare occurrence and may result in delayed diagnosis. We report such a case.

Methods: The baby and his mother's case records were retrospectively analysed. A literature search on atypical presentations of Bart's Hydrops was performed.

Results: A Chinese woman with oligohydramnios and a growth-retarded fetus underwent elective caesarean section at 35 weeks gestation. Her antenatal haemoglobin level was 10.5 g/dL but a Mean Corpuscular Volume (MCV) was not determined. The male infant had an Apgar score of 6 at 5 minutes. He had pallor, hepatomegaly, hypospadias, undescended testes, weighed 1991 g (3rd centile) and was not hydrotic. Despite intensive cardiorespiratory support for presumed Persistent Pulmonary Hypertension of Newborn, he remained hypoxic and acidotic. His low haemoglobin level (11.8 g/dL), low MCV (102 fL), marked anisopoikilocytosis and nucleated red blood cells on the peripheral blood film suggested the possibility of a haemoglobinopathy. Both parents had low MCV values (mother 68 fL, father 70 fL). The baby's haemoglobin electrophoresis showed presence of only Haemoglobin Bart's, Haemoglobin H and embryonic haemoglobins confirming the diagnosis of Bart's Hydrops. Literature search confirmed the association of genitourinary abnormalities with Bart's Hydrops and offered possible pathophysiological reasons for a non-hydrotic presentation.

Conclusion: Alpha thalassaemia carriers need not be anaemic during pregnancy and fetuses with Bart's Hydrops need not be hydrotic. The value of the MCV in identifying carriers and affected cases is demonstrated.

I063/MPC

Newborn Hearing Screening – Improving Programme Performance

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Aim: In 1999, the National University Hospital introduced Newborn Hearing Screening using a single stage strategy of measuring transient evoked otoacoustic emissions (TEOAE). The aim of this study was to measure the improvement to the programme performance resulting from the introduction of a second stage where automated brain stem responses (AABR) are measured.

Methods: Babies are screened within 24 hours of birth using an automated OAE screener. Those with poor responses are rescreened with a semi-automated AABR. Those showing poor responses again are rescreened as outpatients at about 6 weeks' of age. Those not meeting the pass criteria are classified as having been screened positive and are referred to ENT for diagnostic studies.

Results: From April 2003 to March 2004, 2283 (99.6%) of newborns were screened and the 14 who tested positive were referred for diagnostic evaluation. Thirteen were confirmed to have hearing loss, giving an incidence of 5.7/1000 newborns. The incidence of severe/profound hearing loss was 0.88 per 1000 newborns. The specificity and positive predictive value were 99.9 and 93%. With the original single stage strategy, the specificity was 93% and the PPV 2.6%.

Conclusion: The introduction of a second stage of screening by an AABR evaluation has produced a marked improvement in specificity and PPV. This will enhance the acceptability of the screening programme and specifically reduce the inconvenience, costs and anxiety that are consequent to a referral for diagnostic evaluation.

I064/MPC

Current Pattern of Hyperbilirubinaemia in Glucose-6-Phosphate Deficient (G6PD) Male Newborns Diagnosed by Newborn Screening and Hospitalised from Birth

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Aim: G6PD deficient newborns are currently electively hospitalised for 7 days after birth to prevent kernicterus. The aim of this study is to determine the pattern of hyperbilirubinaemia in a recent cohort.

Methods: A retrospective study was performed on G6PD deficient male babies born in the National University Hospital, Singapore between January 1998 and July 2003. Case records were traced for demographic data, serum bilirubin results, incidence and timing of phototherapy and duration of hospitalisation.

Results: There were 146 cases during this 5½-year period. The median duration of hospitalisation was 7 days. Eighteen infants had serum bilirubin levels exceeding 255 µmol/L, but none had levels exceeding 340 µmol/L. Fifty-four infants (37%) underwent phototherapy; 62% of them received phototherapy within the first 72 hours of life, 18% between 72 and 96 hours of life and the remaining 10 babies between 97 and 144 hours of life. Only 2 infants needed re-admission for phototherapy in their second week of life. No case developed kernicterus.

Conclusion: The current strategy of a 1 week elective hospitalisation has proven to be effective in preventing kernicterus. Significant hyperbilirubinaemia in G6PD deficient neonates is mostly in the first 4 days of life. However, we speculate that a 4-day hospitalisation strategy in the absence of clinical risk factors, followed by outpatient surveillance will prove to be as effective.

I065/MPC

Family-Centred Neonatal Intensive Care – Parental Perceptions

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Aim: Contemporary newborn intensive care requires significant participation by parents. Parents traditionally see themselves only as visitors of their baby. The aim of this study was to determine the path leading to the practice of family-centred care.

Methods: A 31-statement questionnaire that addressed parental perceptions of ward practices, health personnel and physical environment of the nursery was administered to 36 sets of parents whose babies were discharged alive from the Neonatal Intensive Care Unit (NICU) between August 2003 and

January 2004. Their responses were scored on a 5-point Likert Scale ranging from Strongly Agree, Agree, Not Sure, Disagree and Strongly Disagree.

Results: Twenty-four parents responded. Their role in decision-making (3.04) and awareness of the doctor-in-charge (3.37) were poorly rated. They clearly preferred more involvement in care activities; e.g. being with their baby during procedures (3.8-3.9) and ward rounds (4.46). Private and more comfortable resting and visiting space was also needed (3.87, 3.62). The nursery was found to be not noisy, bright or anxiety-provoking (2.42, 2.50, 2.71). Rated highly were the knowledge and approachability of the doctors (4.17, 4.08) and nurses (3.71, 4.00).

Conclusion: We have identified areas for improvement in order to achieve family-centred care in the NICU. Physical improvement has begun with the creation of a family room for rest and private communication with health personnel. The results of this study will be shared with other staff members to enrol them in the process of facilitating parental participation in care and identifying the baby's primary physician.

I066/MPC

Traumatic Extradural Haematoma of the Posterior Fossa in Children – Report of 13 Cases

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Aim: Traumatic posterior fossa extradural haematomas (TPFEDH) in children are reported to be relatively uncommon. However, our clinical impression is that they occur fairly frequently. Our aim is to study the clinical presentation and outcomes of children with this type of head injury admitted to our institution.

Methods: Case record and surgical record review of children with this condition admitted to our institution from 1998 to 2003.

Results: Thirteen children with traumatic posterior fossa extradural haematoma were identified. All had a history of mild to moderate blunt trauma to the occiput. The cause of injury in the majority was due to falls. Most of the cases (72%) occurred in young children aged from a few months to 6 years. The clinical presentation was mainly headache, vomiting, and decreasing level of consciousness. The haematomas were diagnosed on CT scanning and all cases underwent surgical intervention. The postoperative outcome in all was good.

Conclusion: Traumatic extradural haematoma of the posterior fossa in children is not uncommon and occurs as a result of a blunt head trauma. Initial loss of consciousness is uncommon and symptoms develop slowly. Most patients presented with high GCS scores. Persistent mild headache, vomiting, and parents' sense of their child's "unusual behaviour" warrants a head CT scan to exclude the diagnosis. Surgery for TPFEDH is safe and the outcome is excellent.

I067/MPC

Traumatic Haematuria Presentation of Congenital Pelviureteric Obstruction

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Aim: Gross haematuria in a paediatric patient following seemingly trivial trauma should arouse the suspicion of a pre-existing abnormality.

Methods: We report a 14-year-old girl with previously asymptomatic and undetected pelvi-ureteric obstruction who first presented with gross haematuria following a minor fall. The diagnosis was established during the index admission by an abdominal CT scan. Our initial treatment was non-operative to allow healing of the laceration and subsequently quantify the renal function and obstruction by follow-up ultrasound and isotope renal scan. Definitive surgical treatment of the PUJ obstruction was achieved by a dismembered (Anderson-Hynes) pyeloplasty.

Results: On follow-up at 6 months, the blood pressure and renal function are normal. A repeat renal scan is planned.

Conclusion: Gross haematuria is an important indicator of severe urinary tract injury. Haematuria following apparently minor trauma should alert the physician to the possibility of a pre-existing abnormality. The presence of incidental genitourinary anomalies in paediatric renal trauma does not seem

to have an impact on recovery from the injury itself. CT scan is recommended as the initial imaging. The management is first directed towards the treatment of the injury. Once the injury heals then the surgical treatment of the abnormality follows standard guidelines. The long-term prognosis is good.

I068/MPC

Anterior Urethral Valves

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Aim: We studied the clinical presentation and management of 4 patients with anterior urethral valves; a rare cause of urethral obstruction in male children.

Methods: One patient was antenatally diagnosed with oligohydramnios, bilateral hydronephrosis and bladder thickening. Two patients presented at 1 and 2 years' of age with poor stream of urine since birth. The oldest patient presented at 9 years with frequency and dysuria only. Diagnosis was established on VCUG (voiding cystourethrogram). All patients had cystoscopic ablation of the valves. One patient had repeated attempts at cystoscopic ablation; he developed a postablation stricture and an open urethroplasty was performed. A bilateral VUJ obstruction was also simultaneously treated by a bilateral ureteric reimplantation.

Results: On long-term follow-up all patients demonstrate a good stream of urine. The renal function is normal. There is mild residual upper tract dilatation in 1 patient who was detected antenatally. Patients are continent and free of urinary infections.

Conclusion: Anterior urethral valves are rare obstructive lesions in male children. The presentation can vary. A severe obstruction would result in antenatal hydronephrosis and renal impairment whereas a lesser grade may present postnatally with mild micturition difficulty. Hence, it is important to routinely evaluate the anterior urethra in any male child with suspected infravesical obstruction. The diagnosis is established by a VCUG and the treatment is always surgical; either a cystoscopic ablation or open resection with urethroplasty depending on the adequacy of urethral support and presence of stricture. The long-term prognosis is usually good.

I069/MPC

Congenital Branchial Cleft Derivative Presenting as an Intrathyroid Abscess

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Aim: The thyroid gland is extremely resistant to acute bacterial infection. Hence the occurrence of such an infection should alert the physician to an underlying pyriform sinus fistula.

Methods: We report a case of an intrathyroid abscess in a 5-year-old boy who presented with a tender thyroid nodule that was resistant to antibiotics. Suspicion of a communication with the oral cavity arose when actinomyces was identified within the fine needle aspirate. A persistent pyriform sinus fistula was confirmed on a barium swallow.

Results: Incision and drainage was not performed. Instead a planned thyroid nodulectomy with excision of the fistulous tract in continuity was performed with perioperative penicillin cover. The penicillin was continued for 6 months.

Conclusion: The most important principles in the management of pyriform sinus fistula are high index of suspicion when presented with a thyroid abscess and complete surgical excision of the entire tract along with the involved thyroid.

I070/MPC

Retroperitoneal Inflammatory Myofibroblastic Tumour

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Aim: Formerly known as inflammatory pseudotumour or plasma cell granuloma, inflammatory myofibroblastic tumour is a distinctive tumour of proliferating myofibroblasts with a background inflammatory infiltrate and low malignant potential.

Methods: A previously well 12-year-old boy presented with fever, right-sided flank pain and loss of weight of 1-month duration. The ESR was raised;

but the white cell count was normal. An abdominal CT scan revealed a right suprarenal mass measuring 3.5 cm without any calcification. He underwent a laparotomy with complete excision of the tumour. Histology revealed an inflammatory myofibroblastic tumour of the retroperitoneum invading the adrenal without any correlates of aggressive behaviour.

Results: The recovery was smooth. Postoperatively, the ESR normalised. No further treatment was given due to the benign nature of the tumour. A follow-up of 6 months did not reveal any recurrence.

Conclusion: Inflammatory myofibroblastic tumour is usually benign with the potential for local recurrence and occasional metastases. Optimal treatment is complete surgical excision.

1071/MPC

Factors in Gastroenteritis Affecting the Duration of Hospitalisation

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Aim: To audit management practices in children hospitalised for gastroenteritis, in particular: investigations performed, mode of hydration, medication usage and the average length of stay (ALOS).

Methods: A retrospective case series of children (1 month to 18 years old) admitted to the National University Hospital for gastroenteritis between 1 January and 31 December 2002 was done. Cases were identified from the hospital database using ICD-9CM coding for gastroenteritis. Logistic regression was used to ascertain the factors influencing hospitalisation greater than 3 days.

Results: 705 inpatient records were analysed. The mean age was 5.0 years + 2.0 years. Admissions occurred at the average of 2.4 days after the onset of symptoms. The main reasons for admission were clinical dehydration (64%), decreased feeding (16%) and abdominal pain (10%). FBC and U&E were done in 90.4% and only 16% had abnormalities. 88% did not receive oral rehydration prior to admission. 83% required intravenous hydration and only 27% were given oral rehydration during the admission. The ALOS was 2.9 days + 3.0 days, with 20% staying longer than 3 days. Logistic regression identified that use of antibiotic and frequent diarrhoea prior to admission, inability to retain feeds, duration on intravenous drip and co-existence of comorbid conditions were independently associated with an ALOS greater than 3 days. The most significant factor was duration of IV hydration (OR = 4.3, 95% CI 3.2-5.8).

Conclusion: To reduce the ALOS, clinicians should consider reducing the duration of intravenous hydration by using more oral rehydration.

1072/MPC

Our Perception and Understanding of Pain in Neonates

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Aim: To determine the perception and knowledge of the house staff (doctors and nurses) with regards to pain in the neonates and to determine their feelings about their role in relieving the neonates' pain.

Methods: A cross-sectional survey was conducted in May 2004. Doctors and nurses of the Neonatal Intensive Care Unit (NICU) were recruited. Information concerning the perception, myths, attitudes and ethics of pain in neonates were obtained using a self-administered questionnaire.

Results: A total of 40 house staff, including nurses, healthcare assistants and doctors were recruited. All of them believe that babies can feel pain. Fifteen (37.5%) think that babies can remember pain. All agreed that doctors and nurses have a responsibility to relieve the neonates' pain. They also feel that the care of the infants in NICU can be improved by implementing good pain management. Only 8 (20%) feel that it will inconvenience them if a pain protocol is implemented, reasons being that it may cause parents/staff conflicts, complications in patient, less attention for the sicker babies and delay in treatment of emergencies.

Conclusion: Most of the staff nursing the babies are aware that babies feel pain and would like to be actively involved in managing the newborns' pain. Only a handful of staff feels that it is inconvenient. It is our goal that with better education, identifying pain will become part of the physical examination and managing it, a part of holistic care for the little ones.

1073/MPC

Quality of Life Before and After Tonsillectomy-Adenoidectomy in Nocturnal Polysomnography Proven Childhood Obstructive Sleep Apnoea

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Aim: The aim was to determine the quality of life (QOL) before and after surgery in nocturnal polysomnography proven childhood obstructive sleep apnea (OSA).

Methods: The validated QOL survey of paediatric OSA, the OSA-18 form, was used in childhood OSA proven by nocturnal polysomnography and who received tonsillectomy with/without adenoidectomy. Cases with underlying craniofacial, metabolic or other abnormalities were excluded.

Results: There were 16 children (median age 6.3 years, range 3.9-15.4 years). The median obstructive apnea index (OAI) was 2.6/H (range 1.0-16.7/H). In 15% of children, OSA had a small impact; in 39% it had a moderate impact, and in 46% it had a large impact on QOL. There were highly significant improvements in global QOL and in most QOL domains (sleep disturbance, physical symptoms, daytime function and caregiver concerns); after curative surgery for OSA. Improvements were largest in the domains of sleep disturbance and caregiver concerns. The OSA-18 severity score (total score) was not related to pre-operative OAI ($r = 0.05$, $P = 0.88$) or BMI ($r = 0.24$, $P = 0.48$). The change score was also not related to pre-operative OAI ($r = 0.06$, $P = 0.86$) or BMI ($r = -0.30$, $P = 0.38$).

Conclusion: There was a moderate to large impact on QOL in 85% of children with nocturnal polysomnography proven OSA. There were highly significant improvements in global QOL and in most QOL domains; after curative surgery for OSA.

1074/MPC

Psychological Distress and Coping in Patients Following Recovery From SARS

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Aim: The physical illness resulting from severe acute respiratory syndrome (SARS) has been a subject of interest and research. Although less attention has been given to the psychological suffering during SARS illness, clinical evaluation suggested a significant level of psychological distress. The aim of this study was to profile the pattern and severity of psychological distress and associated coping strategies, in patients following recovery from SARS.

Methods: One hundred and fifty-four eligible patients were sent questionnaires by post. These included the Hospital Anxiety and Depression Scale, Impact of Event Scale, General Health Questionnaire 12 and COPE questionnaire. We used multiple regression analysis to ascertain the relationship between different coping strategies used and the levels of psychological distress perceived.

Results: We received a response rate of 39.6%. 14.8% and 18.0% of subjects reported at least moderate depressive and anxiety symptoms. 51.7% suffered from moderate post-traumatic stress symptoms. Significant psychological distress, as measured by the GHQ-12, was present in 38.3% of respondents. Patients reported using higher levels of "emotion-focused" coping than "problem-focused" coping strategies. The 3 most commonly used strategies were "Acceptance", "Positive Reinterpretation and Growth" and "Turning to Religion". Greater use of "restraint coping" and "focusing on and venting emotions" was also associated with poorer psychological distress outcomes.

Conclusion: Psychological Distress exists in SARS survivors. These included symptoms of Depression, Anxiety and Post-Traumatic Stress. The use of certain coping strategies was associated with poorer outcome.

I075/MPC**Quality of Life in Schizophrenia: WHOQOL-100 Versus SF-36**
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Aim: Quality of life (QOL) is a critical outcome indicator in healthcare, for which measurement is disease and culture-dependent. The WHQOL-100 and Medical Outcome Short Form-36 (SF-36) are 2 commonly used self-report QOL scales. We aimed to compare these 2 scales against clinician assessment of psychopathology to ascertain their validity in local patients with schizophrenia.

Methods: Fifty stabilised patients with schizophrenia and 50 normal controls were assessed using the WHOQOL-100 and SF-36. Patients were also rated on the positive and negative symptom scale (PANSS).

Results: Both the WHOQOL-100 and SF-36 showed high internal consistency (Cronbach alpha = 0.89 and 0.88 respectively). The domains of the 2 QOL questionnaires, except the social domain, showed significant inter-correlations in both patients and control groups. The WHOQOL-100 domains correlated with PANSS positive, negative and general symptoms, while SF-36 domains correlated only with PANSS depression scores. The domains of both scales, except the WHOQOL-100 spiritual domain, were able to discriminate between patients and controls. Within the patient group, WHOQOL-100 domains differed between those with higher and lower negative symptom and depression scores, while SF-36 domains differed only in patients with higher and lower depression scores.

Conclusion: While both the WHOQOL-100 and SF-36 are valid assessments of QOL in local schizophrenia patients, the WHOQOL-100 appears to better reflect the effect of schizophrenia psychopathology.

I076/MPC**Does Result Presentation Influence Physician Interpretation of Published Research? – Analysis of the Primer 1 Randomised Study**

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Aim: There is good evidence that doctors interpret research poorly, however there has been little investigation as to how interpretation might be improved. Our aim was to determine whether presenting results with increasing levels of information in terms of *P* values (*P*), 95% confidence intervals (CI), and confidence levels (CL) leads to better interpretation.

Methods: Physicians in 2 Singapore teaching hospitals were randomly allocated 1 of 3 questionnaires, each using a different method to present the results of the same 5 artificial studies. The study results were presented with *P*, *P*+CI or *P*+CI+CL depending on the questionnaire. Physicians classified study results depending on conclusiveness, and statistical and clinical significance. Study scenarios and correct answers were based on published reports.

Results: Median age of the 71 participants was 37 (range 25-67) years: 45% were specialists and 55% registrars. 41% had postgraduate training in statistics. 75% read 1-5 journal articles per month, and 18% more than 5. 85% were involved in research, and 69% had first author publications. 32% were journal reviewers/editors. The mean number of correct interpretations (out of 10) for the 3 randomised arms was 6.9 (*P*+CI+CL), 5.5 (*P*+CI) and 5.0 (*P*) (test for trend, *P* = 0.01).

Conclusion: We provide the first randomised evidence that physicians interpret research differently depending on the mode of result presentation. Reporting results with more information in terms of 95% confidence intervals and confidence levels leads to significantly better interpretation by physicians, researchers, journal readers, reviewers and editors compared to *P* values alone.

I077/MPC**Evaluation of Physician Clinical Performance in a New Asian Radiation Oncology Centre: Quality Equipment Does Not Guarantee Quality Practice**

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Aim: A regional (non-Singaporean) Asian radiation oncology centre staffed by nationally-accredited radiation oncologists (ROs) commenced operation in 2003. TCI-Singapore was tasked with auditing radiotherapeutic management received by patients.

Methods: The regional centre wished to remain anonymous. A Singapore RO audited 100 randomly selected patients simulated for radiation by the centre in 2003. Patient charts, radiation prescription, planning CTs, and simulation/verification films were scored using validated criteria.

Results: Median patient age was 48 (range 10-85) years. Primary sites included CNS (22%), head and neck (18%), NSCLC (16%), unknown primary (10%), colorectal (9%) and haematological (6%). Treatment was radical (62%), palliative (32%), benign (2%) and unknown (4%). Deficiencies occurred in documentation, QA and performance criteria. For example, documentation of stage/histology/primary site was inadequate in 38%, portal imaging was unsigned in 60%, and simulation films, plans or prescriptions were unsigned in 48%. Field arrangement was inadequate in 34% of cases, portal films and simulation/verification films differed significantly and were not rectified in 27%, and radiation dose was inadequate in 7%. Specific examples included a nasopharyngeal cancer patient receiving 70 Gy to the whole cervical cord, and a NSCLC patient where treatment missed the target by 10 cm throughout radiotherapy.

Conclusion: A significant proportion of patients audited received suboptimal care, likely resulting in compromised cure/palliation or serious morbidity. This study highlights: 1) formalised training is required in countries providing radiotherapy services, 2) regional and international educational/regulatory bodies, and medical industry should play a greater role in RO training in developing nations, 3) purchasing high-end equipment does not compensate for inadequate education.

I078/MPC**Improving the Interpretation of Clinical Research by Oncologists: Results of the PRIMER 2 Randomised Study**

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Aim: The PRIMER 1 randomised study found research interpretation improved when Singapore physicians were provided with more information. PRIMER 2 was designed to determine which method of result presentation leads to the best interpretation of research.

Methods: Chinese oncologists were randomly allocated 1 of 3 questionnaires, each using a different method to present the results of the same 5 artificial studies. Study results were presented with *P* values + 95% confidence intervals + confidence levels (*P*+CI+CL), *P*+CI, or *P*+CL, depending on the questionnaire. Participants classified studies depending on statistical and clinical significance. Study scenarios and correct answers were based on published examples.

Results: Median age of 225 participants was 34 (range 24-65) years: 58% were specialists and 61% had statistics training. Sixty-four per cent read 1-5 journal articles/month, 32% more than 5. Eighty-four per cent had first author publications; 28% more than 5. Seventeen per cent were medical journal reviewers/editors. The mean number of correct interpretations (out of 10) for the 3 randomised arms was 3.6 (*P*+CI+CL), 2.8 (*P*+CI) and 2.7 (*P*+CL). The odds ratio (OR) of correct interpretation for *P*+CI+CL compared to *P*+CI was 1.4 (*P* = 0.04, 95% CI 1.02-1.96, 98% confidence that *P*+CI+CL is superior), with no difference between *P*+CL vs *P*+CI. *P*+CI+CL was most beneficial for scenarios classified as statistically significant and clinically relevant (OR 2.3, *P* = 0.004), statistically significant (OR 1.7, *P* = 0.002), and conclusive (OR 1.6, *P* = 0.02).

Conclusion: Presenting research results using P+CI+CL leads to better result interpretation compared to P+CI or P+CL. We advocate that medical journals request confidence levels, 95% confidence intervals, and *P* values for endpoint reporting.

1079/MPC

Study on Simultaneous Inter-arm Blood Pressure Measurements in Acute Stroke Patients

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Aim: Accurate determination of blood pressure is crucial in the management of acute stroke patients. Limited research has been done on the inter-arm blood pressure differences in such patients. The aims of our study are to determine whether any such difference exists and whether factors such as severity of stroke and presence of flaccidity or spasticity, have any influence on the blood pressure readings.

Methods: This is a prospective study on patients with a first-ever unilateral stroke of less than a month's duration. Blood pressure measurements were taken simultaneously from both arms using 2 standardised automated blood pressure monitors. Other parameters recorded included handedness, power and tone of the upper limbs as well as the upper limb arm circumference.

Results: Of the 50 patients recruited thus far, 52.9% were males with a median age of 63.4 years. They were enrolled, on average, 2 weeks after stroke onset. In half of them, the affected upper limb was flaccid. The mean difference in inter-arm systolic blood pressure was 2.08 mmHg (95% CI -5.5-1.3) and the mean difference in inter-arm diastolic blood pressure was 1.45 mmHg (95% CI -3.2-1.3). Factors such as inter-arm difference in circumference, tone and power of the affected upper limb did not have any significant effect on the blood pressure readings.

Conclusion: This preliminary study did not show any significant difference in the blood pressure readings between those taken on the normal upper limb as compared to the hemiplegic limb in patients with acute stroke.

1080/MPC

Cost Effectiveness Analysis of Osteoporosis Treatment in the Prevention of Fractures in Singapore

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Aim: To estimate the cost effectiveness of bisphosphonates (Alendronate, Risedronate and Etidronate) or Raloxifene versus no therapy over a 2-year treatment period in patients with osteoporosis and in those with previous fragility fracture.

Methods: A cost-effectiveness analysis using local cost data and clinical data from meta-analysis or randomised controlled trial. The main perspective of the economic evaluation was that of healthcare purchaser. Based on this perspective, only direct healthcare costs were considered in the evaluation. A decision analytic model was used in the evaluation. Five interventions were considered in the decision analytic model in 2 separate groups of patients. A 2-year time horizon was chosen as clinical data about the efficacy of the various interventions considered are available.

Results: The results of the evaluation of the cost-effectiveness of the various interventions showed that it is not cost-effective to treat all patients. However, the analysis performed did not consider indirect and intangible costs in the evaluation process (due to the perspective used in the evaluation). The ranking and magnitude of the incremental cost-effectiveness ratio of the various treatments did not change by the sensitivity analyses using the 95% CI of the efficacy data.

Conclusion: If treatment is indicated, then it would be prudent to consider cyclical etidronate if the risk of hip fracture is low. Raloxifene can be considered if patients will benefit from other non-osteoporosis indication in this group of patients. However, in patients who have high risk of hip fracture, alendronate or risedronate will be more appropriate.

1081/MPC

An Oriental Inception Cohort of Early Rheumatoid Arthritis

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Aim: To obtain a profile of Oriental patients with Rheumatoid Arthritis (RA) with less than 2 years' disease duration treated in a major tertiary centre in Singapore.

Methods: An early RA registry has been established in the department since September 2001. Demographic characteristics, clinical features, therapies, DAS 28, Health Assessment Questionnaire (HAQ) score, Rheumatoid Attitude Index (RAI), ACR Functional Class and SF-36 are entered into a standard protocol.

Results: There was a total of 218 patients with F:M ratio 4.3:1, the majority being Chinese (79%). The overall mean disease duration was 5 ± 5 months (range 0-24) and mean age at diagnosis was 49 ± 12 years. The mean duration of symptoms prior to seeking medical treatment and diagnosis was 6 ± 4 months. The mean DAS 28 score was 4.2 ± 1.5 at recruitment. Most of the patients were in ACR Functional Class 1 (68%). The disability of these patients was moderate: mean HAQ score was 0.6 ± 0.64 . Learned helplessness in this group of patients was moderate with the helplessness subscale of 42 ± 6 . At this early stage of disease 8 patients (3.7%) were unemployed as a result of this illness. The majority of patients (91%) were commenced on low dose oral prednisolone. The disease modifying drugs (DMARDs) prescribed were methotrexate (56%), sulfasalazine (34%) and hydroxychloroquine (8%).

Conclusion: In this cohort of early arthritis patients, significant disability and impaired function were observed and this has important impact on employment. The prospective longitudinal follow-up of this cohort of patients will provide more information about their prognosis and the related predictive factors.

1082/MPC

Diagnostic Value of Anti-cyclic Citrullinated Peptide Antibody in Southeast Asian Patients with Rheumatoid Arthritis

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Aim: To evaluate the sensitivity and specificity of anti-cyclic citrullinated peptide (anti-CCP) antibody in rheumatoid arthritis (RA) patients and to correlate anti-CCP and rheumatoid factor (RF) levels in patients with rheumatoid arthritis (RA), Sjogren's syndrome (SS), systemic lupus erythematosus (SLE) and normal controls. Serum cytokine levels were also compared in patients with or without RF.

Methods: Anti-CCP antibody was measured using ELISA in 59 RA patients, 20 SS, 20 SLE and 47 normal controls. The revised 1987 American College of Rheumatology criteria for RA, 1982 revised criteria for SLE and the European classification criteria for SS were used. Sensitivity and specificity of anti-CCP level were compared between various rheumatic diseases and normal controls.

Results: The sensitivity and specificity of anti-CCP antibody were 62.3% and 92.1% respectively whereas the sensitivity and specificity of RF were 82% and 65% respectively. Unlike SLE, SS and normal controls, there was a strong correlation between IL1 and IL6 in RA patients ($\rho = 0.72$, $P < 0.001$). Significant correlation was found between anti-CCP and RF ($\rho = 0.58$, $P = 0.0004$). Neither RF nor anti-CCP correlated significantly with IL1 or IL6. Significant correlation was found between TNF and anti-CCP ($\rho = 0.52$, $P = 0.002$) as well as TNF and RF ($\rho = 0.55$, $P = 0.001$).

Conclusion: Anti-CCP was more specific but less sensitive than RF in our local RA population and therefore can be used to distinguish RA from SLE and SS. A significant correlation was also found between anti-CCP, RF and TNF.

I083/MPC**What Does Patient Global Assessment of Health Measure When SLE Patients Are Asked to Evaluate Their Own General Health?**KO KONG¹, BYH THONG¹, TY LIAN¹, ET KOH¹, YK CHENG¹, HS HOWE¹, AL ET¹¹Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital, Singapore

Aim: Patient global assessment of health (PatGA) is frequently used as a summary index of disease impact on health. We sought to elicit important factors associated with poor health in the PatGA in systemic lupus erythematosus (SLE) patients.

Methods: SLE patients (fulfilling ACR criteria or with histologic evidence of SLE) were consecutively enrolled into a prospective study cohort with demographic, clinical and laboratory data, SLEDAI and SLAM disease activity indices, the SLICC damage index, and quality of life index (SF-36) being collected. Physician global assessment of disease activity (PGA) and patient estimation of overall health (PatGA) were scored using visual analogue scales.

Results: A total of 460 patients were enrolled, mainly Chinese (78.9%) females (90.9%), with mean age 41 ± 13 years, and median disease duration 7.1 (2.8-13.7) years, the median for SLEDAI score was 2 (0-4), SLAM score 2 (0-4), PGA 9 (1-23) and PatGA 23 (11-43). SF-36 median scores for physical function was 80, role-physical 100, social function 87.5, role-emotional 100, mental health 68, general health 57, vitality 60 and bodily pain 74. PatGA correlated weakly with PGA, SLEDAI and SLAM (ρ : 0.16-0.21) but significantly with most SF-36 domains (ρ : 0.23 to 0.50). The main factors affecting PatGA were the vitality and helplessness scale when adjusted for age, gender, race and disease duration.

Conclusion: PatGA reflects the effect of SLE on energy level and the helplessness in disease state and tended to relate more with quality of life than physical effects of disease.

I084/MPC**Early Oriental Lupus Cohort – A Prospective Follow-up of Newly Diagnosed Lupus Patients of Oriental Ethnicity**KO KONG¹, BYH THONG¹, TY LIAN¹, WG LAW¹, YK CHENG¹, HS HOWE¹, AL ET¹¹Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital, Singapore

Aim: Prospective cohort studies of systemic lupus erythematosus (SLE) from Asia are few. Thus, we sought to prospectively study our cohort of early (disease duration <3 years) Oriental SLE patient.

Methods: SLE patients who fulfilled ACR criteria were consecutively enrolled into a prospective study cohort with 4-monthly assessments of demographic, clinical and laboratory data, SLEDAI and SLAM disease activity indices, the SLICC damage index, and quality of life index (SF-36).

Results: Out of 460 patients, 118 had early lupus (48% newly diagnosed), mainly Chinese (72.0%) females (92.4%), with mean age 35 ± 13 years, disease duration 14 ± 12 months; although 7% had <4 ACR criteria at recruitment. The median SLEDAI score was 4 (range 2-8), SLAM score 3 (1-6), and SLICC 1 (0-1). The commonest manifestations were mucocutaneous (75.6%), haematological (67.4%), and constitutional (53.3%). Major internal organ involvement was frequent (53.3%: 28.9% renal 22.2% neuropsychiatric). There were 83.5% with antibodies to dsDNA, 90.7% antinuclear antibodies and 71.1% hypocomplementaemia. Compared to the overall cohort, early lupus patients had more cutaneous and less cardiac and renal involvement, higher average disease activity, they were older at diagnosis (34 ± 1 vs 31 ± 1 years, $P = 0.04$), and more adversely affected in role-physical, role-emotional and social function domains of the SF-36. Corticosteroids (74.8%), hydroxychloroquine (75.2%) and immunosuppressants (34.1%, mainly cyclophosphamide and azathioprine) were the main drugs used.

Conclusion: Major organ involvement is common in the early lupus cohort. Limitation of role and social functioning are adversely affected with the initial diagnosis of SLE.

I085/MPC**Severity of Anaemia Reflects Disease Activity But Not Associated with Damage Accrual in Systemic Lupus Erythematosus (SLE)**

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Aim: Anaemia has been a non-specific indicator of disease activity of SLE in the absence of haemolytic anaemia. We seek to investigate the association of the severity of anaemia and disease activity in SLE patients.

Methods: SLE patients (fulfilled ACR criteria or had histologic evidence) were recruited. Demographic, clinical and laboratory information, disease activity indices (SLEDAI, SLAM) and damage index (SLICC) were collected. Physician global assessment (PGA) of disease activity was scored using visual analogue scale. Severity of anaemia were expressed as 3 categories: normal (hematocrit, Hct >35%), mild (Hct: 30%-35%), and significant (Hct <30%).

Results: 460 patients had been recruited with mean age 41 ± 13 years, median disease duration 7.1 (range 0-36) years. The median SLEDAI score was 2 (0-39) corresponding with SLAM: 2 (0-28) and PGA: 9 (0-100). The median SLICC was 1 (0-8). Overall, 25% of patients had anaemia at recruitment, with 6% having significant anaemia. Anaemia is significantly more commonly found in those with renal involvement (29.9% vs 18.6%, $P = 0.02$). Significantly higher disease activity, whether assessed using SLEDAI, SLAM or PGA, was noted in worse anaemia ($P < 0.001$). This was not observed in the cumulative damages.

Conclusion: Anaemia is common in SLE and associated with higher disease activity but not cumulative damage. It is a useful and simple tool in assessing SLE disease activity, in particularly among those with renal involvement when haemolytic anaemia is excluded.

I086/MPC**Relationship Between Psychosocial Factors and Risk of Developing Eating Disorders in Young Females**TF HO¹, HC LIM¹, T JAYABASKAR¹, EL LEE², PH LIOW³, S CHENG⁴¹Physiology, Faculty of Medicine, National University of Singapore, Singapore, ²Department of Medicine, Singapore General Hospital, Singapore, ³Department of Medicine, Alexandra Hospital, Singapore, ⁴Department of Child and Adolescent Psychiatry, Institute of Mental Health/Woodbridge Hospital, Singapore

Aim: The aim of this study was to identify the social and psychological risk factors which may predispose young females to develop Eating Disorders (ED).

Methods: A set of self-administered questionnaires consisting of an Eating Attitude Test (EAT), an Eating Disorder Inventory (EDI) and a socio-demographic questionnaire was administered to 4466 young females (12-26 years old; 79% Chinese, 11% Malays, 7% Indians, 3% Others). Based on cutoff values of 29 for EAT and/or 14 for EDI-Drive for Thinness (EDI-DT) subscale, respectively, subjects were categorised into either "Normal" (NM) or "At Risk" (AR) of ED. Psychosocial variables that were univariately associated with AR were further analysed using multivariate logistic regression to identify predictors of AR.

Results: Prevalence of AR was 7.4% ($n = 329$). Seven psychosocial variables were identified as significant predictors of AR. In particular, odds ratios of factors like desiring for BMI in underweight range, has ever been on diet and family history of psychiatric illness were significantly high at 2.07, 5.38 and 1.99, respectively. The other factors include items in the EDI subscales relating to bulimia, perfectionism, interoceptive awareness and body dissatisfaction (odds ratios 1.24, 1.12, 1.10 and 1.11, respectively).

Conclusion: Several psychosocial factors were found to be significant independent predictors of increased risk for eating disorders in young females. These factors had significantly high odds ratios ranging from 5.38 to 1.10.

1087/MPL

Plasma Risperidone Concentrations and Clinical Response in Patients with First PsychosisS VERMA¹, CH TAN², YH CHAN³, SA CHONG¹¹Department of Early Psychosis Intervention, Institute of Mental Health/Woodbridge Hospital, Singapore, ²Department of Psychological Medicine, National University of Singapore, Singapore, ³Clinical Trial Epidemiology Research Unit, Singapore**Aim:** To assess the relationship between plasma concentrations of risperidone and its active 9-hydroxy metabolite (9-OH-risperidone) and clinical response in patients with first-episode psychosis.**Methods:** Nineteen patients with first-episode psychosis admitted to the Early Psychosis Intervention Programme in Singapore, who were started on risperidone were recruited for the study. These patients were evaluated at baseline and weeks 2, 6, and 12. Psychopathology was measured using the Positive and Negative Scale for Schizophrenia (PANSS) and assessment for side effects was carried out using the Simpson-Angus Scale (SAS) and Barnes Akathisia Rating Scale (BARS). Steady-state plasma concentrations of risperidone and 9-OH-risperidone were measured at week 12 using a specific HPLC assay.**Results:** There was a significant correlation between plasma concentrations of risperidone and 9-OH-risperidone as well as between risperidone dosage and plasma concentrations of 9-OH-risperidone. Responders (PANSS reduction >40%) had significantly higher plasma levels of the active moiety than the non-responders ($P = 0.022$). A curvilinear relationship between active moiety and PANSS (%) improvement was observed. Patients with higher PANSS amelioration showed RSP + 9-OH-RSP plasma levels ranging from 15 to 25 ng/mL.**Conclusion:** Therapeutic blood level monitoring can be an aid in managing complex or refractory patients with psychosis.

1088/MPL

FLT3 Mutations Are Distinctly Rare and Do Not Appear to Have Prognostic Significance in a Local Cohort of Patients with Myeloid MalignanciesLG LAU¹, WJ CHNG¹, LK TAN¹, BMF MOW¹, ESC KOAY², TC LIU², YK KUEH³¹Department of Haematology-Oncology, National University Hospital, Singapore, ²Department of Laboratory Medicine, National University Hospital, Singapore, ³Department of Medicine, National University Hospital, Singapore**Aim:** To investigate incidence and prognostic significance of FLT3-activating mutations in a population with myeloid malignancies.**Methods:** Patients with newly-diagnosed myeloid malignancies at the National University Hospital from 2000 to 2002 were identified. The diagnostic bone marrow aspirates were sent for polymerase chain reaction studies for internal tandem duplication (ITD) mutations and point mutations of codon 835 of the FLT3 gene. All new patients after 2002 were prospectively investigated. Clinical and histo-cytogenetic data were collected and analysed with the FLT3 mutation data.**Results:** A total of 87 patients with myeloid malignancies between 2000 and 2003 were studied. Of these, 13 were positive for FLT3 mutations (incidence: 14.9%) - 2 (2.3%) point and 11 (12.6%) ITD mutations. The breakdown of the subtypes of myeloid malignancies was: 2 (15.4%) M1, 5 (38.5%) M2, 1 (7.7%) M3, 2 (15.4%) M4 and 3 (23.1%) M5. The breakdown of cytogenetic abnormalities was: 8 (61.5%) normal, 1 (7.7%) good, 3 (23.1%) intermediate and 1 (7.7%) adverse cytogenetics. Both univariate and multivariate analyses for overall (OS) and disease-free survival (DFS) failed to show FLT3-activating mutations as a significant prognostic factor in the whole cohort. It was also not a significant prognostic factor for OS and DFS when analyses were confined to patients with intermediate and normal cytogenetics.**Conclusion:** FLT3-activating mutations are distinctly rare and do not appear to have prognostic significance in a local cohort of patients with myeloid malignancies.

1089/MPL

Circulating Dengue Serotypes in SingaporeA WILDER-SMITH¹, S YOKSAN², A EARNST³, R SUBRAMANIAM¹, NIPATON¹¹Department of Infectious Disease, Tan Tock Seng Hospital, Singapore, ²Mahidol University, Bangkok, Thailand, ³Department of Clinical Epidemiology, Tan Tock Seng Hospital, Singapore**Aim:** Dengue virus is a flavivirus with 4 virus serotypes, transmitted by mosquitoes of the genus Aedes. Dengue virus infection of all 4 virus serotypes causes a spectrum of illness ranging from asymptomatic to fatal haemorrhagic disease. Co-circulation of various serotypes is thought to increase the emergence of dengue haemorrhagic fever (DHF). We conducted a seroepidemiological study to determine the extent to which the Singapore population has been exposed to dengue and to determine the proportion of circulating virus serotypes.**Methods:** Healthy volunteers between the age of 18 and 30 were recruited consecutively. Dengue antibodies were measured with PanBio Dengue IgG ELISA. The plaque reduction neutralisation assay was performed to determine the serotype.**Results:** Of the 164 enrolled subjects, 39 (23.8%) had a positive dengue serology. The odds ratio of dengue seroprevalence increased with age, and was much higher for foreigners (75%) from SE Asia compared with Singaporeans (18%). The prevalence of dengue virus type 1 was 24%, for type 2: 23%, for type 3: 26% and for type 4: 18%. Of the 39 positive results, 28 (57%) were due to infection with all four viruses, 7 (14%) to 3, and 12 (25%) to 1.**Conclusion:** All 4 dengue serotypes circulate in Singapore, and a large proportion of the adult population in Singapore is co-infected with more than 1 dengue serotype. In spite of circulating multiple types, the DHF rate is low in Singapore, suggesting that the immune enhancement hypothesis is not the only explanation for the development of DHF.

1090/MPL

Identification of HER Proteins in Breast Cancer Using ProteomicsDH ZHANG¹, LK TAI², LL WONG³, SK SETHI¹, ESC KOAY⁴¹Department of Laboratory Medicine, National University Hospital, Singapore, ²Department of Pathology and Laboratory, National University Hospital, Singapore, ³Oncology Research Institute, National University of Singapore, Singapore, ⁴Department of Pathology and Laboratory, National University of Singapore, Singapore**Aim:** Background: The HER-2/neu oncogene is a member of the c-erbB family of receptor tyrosine kinases and is involved in cell growth regulation, cell proliferation, invasion and metastasis. HER-2/neu overexpression also confers resistance to certain chemotherapeutic regimens and therefore results in the poor clinical outcome for the patients with HER-2/neu overexpressing breast carcinoma. Aims: To identify the proteins strongly associated with and/or involved in HER-2/neu-modulated molecular events and potentially used for diagnostic or therapeutic purposes.**Methods:** The proteins from HER-2/neu positive tumours and proteins from HER-2/neu negative tumours were separated using two-dimensional (2-D) gel electrophoresis over a pH range of 3 to 10, and differentially expressed proteins were digested and analysed by tandem matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF)-TOF MS/MS. Protein identities were analysed by searching against the NCBI protein database, using Mascot software.**Results:** Twelve proteins with high statistical reliability were identified. They include 14-3-3, Hsp27, triosephosphate isomerase I, phosphoglycerate kinase I, phosphopyruvate hydratase, fatty acid synthase, cathepsin D, haptoglobin, prollyl 4-hydroxylase (beta-subunit), glyoxalase I, annexin II (type I) and PRO2675. The enhanced expression of Hsp27, cathepsin D and triosephosphate isomerase I were further verified by Western blot, reverse-phase protein array and tissue microarrays.**Conclusion:** HER-2/neu overexpression increases the glycolytic metabolism, extracellular matrix degradation and cell protection from stress. The up-regulation of these identified proteins may play a role in the HER-2/neu-mediated tumorigenesis, and are potential therapeutic targets in breast cancer.

I091/MPL**Reproducibility of Measurement for Deep Frozen Serum Procalcitonin PCT Concentration Compared with Fresh Serum Samples of CABG Patients**HD LUO¹, MD COSTA², CTT TAN², CN LEE²¹Department of Medicine, National University Hospital, Singapore,²Department of Cardiac, Thoracic and Vascular Surgery, National University Hospital, Singapore

Aim: Procalcitonin (PCT) has been proposed as an early and specific marker of bacterial infection recently. The influence of cardiopulmonary bypass on production of PCT must be assessed before considering this molecule as a valuable marker of infection after bypass surgery. Before starting the large sample serum test, it is necessary to assess the reproducibility of frozen samples' test results compared with fresh ones.

Methods: Fifty fresh serum samples were obtained from 10 CABG patients who gave informed consent to join in this study at 5 different time points preoperatively and postoperatively. Each fresh sample had 2 aliquot. One was sent to the lab for PCT test at once, the other was deeply frozen and stored in -80°C VIP freezer for 3 months. Each frozen aliquot was sent to the lab for PCT test after 3-month storage.

Results: There is no significant difference in PCT results between fresh samples (Mean: 8.63 ± 12.19 , range: 0.06 to 45.23); and frozen samples (Mean: 8.29 ± 11.92 , range: 0.06 to 47.52) ($P = 0.2$). The results from fresh samples were correlated significantly with the results from frozen samples ($r = 0.987$, $P = 0.0005$). Reliability Coefficients Alpha = 0.9935.

Conclusion: Compared with fresh serum samples, the measurement for PCT concentration in deep frozen serum has a good reproducibility in CABG patients. PCT test results from massive deep frozen samples might be reliable.

I092/MPL**A Report of the Peroxisome Proliferator Receptor- γ 2 Pro12Ala Polymorphism in Chinese Subjects with Type 2 Diabetes Mellitus and Nephropathy**FYA KOH¹, T GOH², XH XU³, SC LIM¹, CF SUM¹¹Department of Medicine, Alexandra Hospital, Singapore, ²Diabetes Centre, Alexandra Hospital, Singapore, ³Clinical Research Unit, Alexandra Hospital, Singapore

Aim: Peroxisome proliferator-activated receptor- γ 2 (PPAR- γ 2) is expressed predominantly in adipose tissue where it plays an important role in adipocyte differentiation. The Pro12Ala polymorphism in PPAR- γ 2 results in reduced transcriptional activity of PPAR- γ 2 and may be associated with improved insulin sensitivity. Studies of this polymorphism in diabetic nephropathy (DN) have been inconsistent, with conflicting reports of the Ala allele conferring protection from or susceptibility to DN. We explored this polymorphism in a group of Chinese patients with Type 2 Diabetes mellitus (T2DM) and aimed to determine the relationship of the Ala allele with DN.

Methods: A case-control study was conducted in Chinese patients with T2DM where cases ($n = 114$) had proteinuria (urinary total protein >0.5 g/day or Urine albumin/creatinine ratio >500 μ g/mg) OR persistent elevation in serum creatinine while controls ($n = 76$) had T2DM for at least 10 years with urine albumin/creatinine ratio <50 μ g/mg AND normal serum creatinine. Genotyping was carried out using standard polymerase chain reaction and restriction fragment length polymorphism followed by electrophoretic resolution.

Results: The frequency of the Ala allele was 7.5% in cases and 5.9% in controls. There was no statistically significant difference between cases and controls in allele distribution ($\chi^2 = 0.14$, $P = 0.708$).

Conclusion: The PPAR- γ 2 Pro12Ala polymorphism was not associated with DN in our study cohort.

I093/MPL**A Novel Diagnostic DNA Chip: Multiplex Allele-specific Primer Extension Strategy for Detection of 12 Drug-metabolising Gene Polymorphisms**Y LU¹, SKY KHAM¹, A YEOH²¹Department of Paediatrics, National University of Singapore, Singapore,²Department of Paediatrics, National University Hospital, Singapore

Aim: Polymorphisms in drug-metabolising genes can both increase toxicity from chemotherapy and improve treatment outcome. To obtain the optimal therapeutic outcome, a patient's genetic profile should be defined so that the correct dosage can be administered. Unfortunately in most clinical laboratories, genetic tests can be only carried out individually for different mutation, using various PCR protocols or restriction enzymes. This severely limits its application in practice. We therefore aimed to develop a microarray-based platform to simultaneously genotype 12 polymorphisms in 6 important genes (TPMT, NQO1, CYP1A1, CYP2D6, MTHFR and GSTP1) in childhood ALL.

Methods: Two allele-specific primers were designed to interrogate each polymorphism and all primers for wild-type or mutant are pooled to obtain 2 mixtures. Eleven DNA fragments spanning 12 polymorphic sites in 6 genes were amplified in separate tubes under identical PCR conditions, pooled, purified and then subjected to multiplex allele-specific primer extension using 2 primer mixtures respectively. Only fully matched primers were extended with TAMRA-labelled ddNTP. The products were subsequently loaded on DNA chip and extended primers were captured by corresponding oligo tags. The fluorescence then disclosed the genotype in the imaging system. The feasibility was initially validated using plasmids and 15 cord blood DNA.

Results: For each polymorphic site studied, homozygosity showed fluorescence from only 1 of 2 primers while signals from both indicated heterozygosity. Our preliminary test achieved 100% accuracy.

Conclusion: We successfully demonstrated the validity of this novel genotyping strategy which has great potential to become a simple and reliable platform for the genetic test.

I094/MPL**Clinical Significance of Thiopurine S-methyltransferase (TPMT) Mutations – Results of the Screening in a Multiracial Asian Patient Population with Leukaemia**CSN KWOK¹, SH TEO¹, SKY KHAM¹, H ARRIFIN², HP LIN³, TC QUAH¹, A YEOH¹¹Department of Paediatrics, Faculty of Medicine, National University of Singapore, Singapore, ²Department of Paediatrics, University of Malaya Medical Centre, Malaysia, ³Department of Paediatrics, Subang Jaya Medical Centre, Malaysia

Aim: Thiopurine S-methyltransferase is central to the breakdown of thiopurine drugs important in leukaemia. In patients with mutations and thus reduced activity, thioguanine accumulation leads to prolonged myelosuppression and an increased risk of second cancers. Previously, we reported the lower incidence of TPMT carriers in the Singaporean compared to Caucasian population. We now report our TPMT study on a large group of Singaporean/Malaysian children with leukaemia.

Methods: DNA was obtained from 334 children with *de novo* or relapse precursor-B ALL ($n = 273$), T-ALL ($n = 20$) and AML ($n = 41$). TPMT genotyping was carried out using restriction-length fragment polymorphism PCR (RFLP-PCR) for the *3A, *3C and *6 mutations.

Results: Overall, after excluding Caucasians ($n = 4$), mutation-carriers were detected at similar frequencies (4.9%) with our population study (4.5%). Interestingly, Indians exhibited a higher frequency of 10% (2/21), all *3C mutations rather than the previously reported *3A in Southwest Indians. *3A mutations were detected mainly in Caucasians ($n = 2/4$), except for 1 Chinese (his mother was also a carrier). *3C is the predominant mutation in Asians (Chinese, Malay, Indians). An extremely rare mutation reported only once in a Korean, *6, was detected in 2 Chinese (2/191; 1%).

Conclusion: TPMT *3C and *6 appear to be the most important TPMT mutants in an Asian population, unlike in Caucasians. It is worthwhile to correlate the toxicity of thioguanine therapy in these groups of patients to determine the optimal range of drug dosing.

I095/MPL**Cyclooxygenase-2 Expression in Nasopharyngeal Carcinoma: Immunohistochemical Findings and Potential Implications**

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Aim: Cyclooxygenase-2 (COX-2), an inducible prostaglandin synthase,

participates in inflammatory and neoplastic processes. It is expressed by various tumours and contributes to carcinogenesis. Notably, COX-2 inhibitors appear to have tumour suppression effects and are being evaluated in clinical trials. Nasopharyngeal carcinoma (NPC), a common tumour in parts of Asia, carries significant morbidity and mortality. Although COX-2 has been studied in other cancers of the head and neck, there is paucity of data on its expression in NPCs. We seek to study COX-2 expression in NPC and to discuss potential implications.

Methods: Eighty-six cases of NPC diagnosed during 1997 to 2000 were reviewed. COX-2 immunohistochemistry and semi-quantitative assessment of expression in nasopharyngeal biopsies were performed.

Results: Histologically, 92% of NPCs were undifferentiated, 7% were non-keratinizing and 1% was keratinizing. 47% of NPCs had adjacent epithelium that was dysplastic. COX-2 expression was noted in 70% of NPCs, 38% of dysplastic epithelium and only 4% of normal epithelium ($P < 0.005$).

Conclusion: Our study affirms predominance of undifferentiated NPCs and the rarity of the more radio-resistant keratinizing subtype. COX-2 expression is more commonly observed as nasopharyngeal epithelium progresses from normal to dysplastic to carcinomatous. This suggests that COX-2 contributes to the multi-step process of NPC carcinogenesis. COX-2 represents a therapeutic target for COX-2 inhibitors and there is, thus, a basis for the further investigation of this adjuvant treatment modality for NPC. Remarkably, COX-2 inhibitors are known to potentiate the anti-tumour effects of radiotherapy, the latter being the primary treatment of NPCs.

1096/MPL

Urine Cytology: Are ThinPrep™ Smears Superior to Cytospin Smears? – A Comparative Study of 120 cases

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Aim: The ThinPrep technique is gaining popularity in both gynaecologic and non-gynaecologic cytology, including urine specimens. In this study, we evaluated the cell distribution, cell preservation, stain distribution and background of the smears as well as the diagnostic accuracy of ThinPrep Processing in comparison to the conventional Cytospin technique in urinary cytology.

Methods: Retrospective, randomised and double-blinded evaluation of 120 voided or instrumented urine samples, prepared by both ThinPrep processing and duplicate slides Cytospin preparation were done. For each case, 3 slides were reviewed – 1 ThinPrep and 2 cytospin smears. Each parameter was scored using a semi-quantitative score of 1 to 3.

Results: Quality of cellular distribution, cell preservation and slide background were better with Cytospin preparation ($P < 0.001$), however, there was no significant difference in stain distribution. Sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were higher for Cytospin technique (85.7%, 92.3% and 71.4%) than ThinPrep samples (66.7%, 88.9% and 62.5% respectively). However, specificity of both techniques remain the same.

Conclusion: In practice, the Cytospin smears are of better quality than those prepared by the ThinPrep™ technique. Although ThinPrep and Cytospin smears result in similar diagnostic accuracies in negative cases, the ThinPrep method was not found to be superior to Cytospin smears in diagnosing positive urinary cytology.

1097/MPL

Implementing Molecular Diagnostics in the Diagnostic Laboratory Rapid Identification of Candida Species in Blood Cultures

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Aim: Candidaemia is increasing, particularly in patients undergoing treatment for cancer and immune modulation after organ transplantation. The choice of antifungal chemotherapy depends on the species. From the time that a blood culture is shown to have a yeast in it, the laboratory may take 4 days to issue a final identification. Meanwhile clinicians may choose to use amphotericin which shows renal toxicity, is unpleasant for the patient and has to be

administered intravenously. Alternatives such as the azole drugs can be given orally and are more acceptable to patients but are not active against all candida species. A rapid identification of candida species would help tailor therapy sooner, to the patient's advantage. **Aim:** To use PCR methods to identify yeasts to the species level and report the result the same day that we see a 'yeast' in the blood film.

Methods: We reviewed the literature, optimised and then validated a multiplex PCR system for use with blood cultures.

Results: We achieved 100% sensitivity and specificity.

Conclusion: This can be used in daily practice. Justification of the cost is not easy. Investment in this technology can be expected to improve outcome, reduce length of stay and reduce other investigations. While the laboratories bear the burden of introducing expensive technology the benefits are seen elsewhere. The 'silo' view of accounting has to be broken down to make the best use of modern technology to help our patients effectively and efficiently.

1098/MPL

Accuracy of the Various Iron Parameters in the Prediction of Iron Deficiency in a Tertiary Hospital

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Aim: Iron parameters like serum ferritin and iron saturation are routinely used in diagnosing iron deficiency. However, these tests are influenced by many factors. We aimed to review the accuracy of iron studies among inpatients in a single institution.

Methods: From 1997 to 2002, bone marrow aspirate samples which had concurrent iron studies done were analysed. Accuracy of the various iron parameters was analysed using ROC curves. Iron parameters which discrepantly predict the presence of iron deficiency were studied.

Results: Among 92 bone marrow samples, 58, 86 and 83 had a concurrent serum ferritin, serum iron and % iron saturation respectively. Serum ferritin is the best marker for predicting the presence of iron deficiency. This is followed by % iron saturation and lastly by serum iron. A serum ferritin of < 0.0001 .

Conclusion: A serum ferritin of < 60 ng/mL or a % iron saturation of $< 7\%$ is highly predictive of iron deficiency. In discrepant cases, the % iron saturation is most likely the spurious result.

1099/MPL

Resveratrol Inhibits Drug-induced Apoptosis in Human Leukaemia Cells by Creating an Intracellular Milieu Nonpermissive for Death Execution

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Aim: Efficient apoptotic signalling is a function of a permissive intracellular milieu created by a decrease in the ratio of superoxide to hydrogen peroxide and cytosolic acidification. Resveratrol (RSV) triggers apoptosis in some systems and inhibits the death signal in others.

Methods: Techniques employed included western blotting, various fluorescent and colorimetric assays.

Results: Exposure of human leukaemia cells to low concentration of RSV (4–8 μ M) inhibits caspase activation, DNA fragmentation and translocation of cytochrome-c induced by hydrogen peroxide or anticancer drugs C2, vincristine and daunorubicin. Interestingly, at these concentrations, RSV induces an increase in intracellular superoxide and inhibits drug-induced acidification. Blocking the activation of NADPH oxidase complex neutralised RSV induced inhibition of apoptosis. Furthermore, our results implicate intracellular hydrogen peroxide as a common effector mechanism in drug-induced apoptosis that is inhibited by preincubation with RSV. Interestingly, decreasing intracellular superoxide with NADPH oxidase inhibitor diphenyliodonium reversed the inhibitory effect of RSV on drug-induced hydrogen peroxide production.

Conclusion: We show that low concentrations of RSV inhibit death signalling in human leukaemia cells via NADPH oxidase-dependent elevation of intracellular superoxide that blocks the mitochondrial hydrogen peroxide production, thereby resulting in an intracellular environment nonconductive for death execution. These findings have significant clinical implication

given the heightened interest in the potential use of RSV in combination chemotherapy regimens. Due to the low bioavailability of RSV, our data suggest death-inhibitory activity of RSV in leukaemia cells at doses that may be relevant physiologically.

I100/MPL

The Effect of Airway Administration of Lignocaine on the Murine Model of Allergic Asthma

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Aim: Lignocaine has been reported to be effective in the treatment of asthma in humans. We sought to investigate the effects of lignocaine on airway inflammation and airway hyper-responsiveness (AHR) in a murine model of allergic asthma.

Methods: On days 0 and 14, 6- to 7-week-old BALB/c mice were sensitised by intraperitoneal injection of ovalbumin (OVA), followed by intranasal challenge OVA on days 14 and 25 to 28. Intranasal lignocaine (1 mg/kg) was administered 1 hour before the last 4 challenges. On day 29, AHR was determined with whole-body plethysmography. Subsequently, the mice were sacrificed. Bronchoalveolar lavage (BAL) and histological sections of lungs were obtained.

Results: Treatment with lignocaine caused a significant reduction in the AHR. There was also an increase in eosinophil and decrease in macrophage numbers in the BAL. Histologic sections showed a decrease in the tissue eosinophilia.

Conclusion: Treatment with lignocaine reduced the severity of AHR with modest effect on the airway inflammatory cellular infiltrate.

I101/MPL

Successful Use of Human Serum for the Culture of Human Melanocytes Opening New Doors in the Treatment of Vitiligo

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Aim: Traditionally, fetal bovine serum (FBS) is used to culture melanocytes. However, FBS, or its substitute, presents risks of foreign protein contamination as well as transmission of viral or prion-related diseases if used in culture of cells intended for human transplantation. Human serum has been used to culture osteoblast but has never been used for the culture of melanocytes. This study aims to evaluate the use of human serum, as compared to FBS substitute, UltrosorG, in the culture of melanocytes.

Methods: Suction blisters were induced from volunteers, trypsinised and cultured in T25 flasks. At confluence, 1 x 10⁴ melanocyte cells were plated onto multiwell plates and cultured for 11 days in medium supplemented with human serum at different concentrations, 2% UltrosorG and medium without serum substitute. The cells were counted using haemocytometer at Days 4, 8 and 11.

Results: Ten sets of melanocytes from different volunteers were cultured. All cultured melanocytes were able to grow well in media supplemented with human serum. There was no significant difference between proliferations of melanocyte in 5% human serum as compared to 2% UltrosorG. However, 10% and 20% human serum resulted in a faster rate of cell proliferation than UltrosorG.

Conclusion: Melanocyte cells can be cultured using human serum, in place of FBS, for transplantation onto vitiligo patients. Culture media containing more than 5% human serum resulted in a faster increase in the number of melanocytes when compared to 2% UltrosorG.

I102/MPL

The Ras Kinase Pathway Inhibitor and Likely Tumour Suppressor Proteins Sprouty 1 and Sprouty 2 Are Deregulated In Breast Cancer

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Aim: Sprouty (Spry) proteins were found to be endogenous inhibitors of the Ras/MAP kinase pathway that play an important role in the remodelling of branching tissues in development. We investigated Spry expression levels in various cancers and found that Spry1 and Spry2 were consistently down-regulated in breast cancers.

Methods: Real time PCR, *in situ* hybridisation, immunohistochemistry, proliferation assays, colony forming assays, *in vivo* xenograft nude mice assays.

Results: Spry1 and 2 were expressed specifically in the luminal epithelial cells of breast ducts, with higher expression during stages of tissue remodelling when the epithelial ducts are forming and branching. The abrogation of endogenous Spry activity in MCF-7 cells by the over-expression of a previously characterised dominant negative mutant of Spry, hSpry2Y55F resulted in enhanced cell proliferation *in vitro*. hSpry2Y55F stably expressing cells also formed larger and a greater number of colonies in the soft-agar assay. An *in vivo* nude mice assay demonstrated a dramatic increase in the tumorigenic potential of hSpry2Y55F stable cells.

Conclusion: The consistent down-regulation of Spry1 and 2 in breast cancer and the experimental evidence utilising a dominant-negative hSpry2Y55F indicate that Spry proteins may actively maintain tissue integrity that runs amok when their expression is decreased below normal threshold levels. This supports a previously unrecognised role for Sprys in cancer development.

I103/NUR

Family Coping with Bereavement – The Perceptions and Role of Nurses in the Emergency Department

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Aim: Death occurs frequently in the Emergency Department (ED), leaving behind bereaved families. Bereavement care for these families is an un-addressed issue. This study aims to determine the perceptions of ED nurses concerning bereavement care provision in the ED.

Methods: A survey was conducted from 17 February to 28 February 2003 using a self-administered questionnaire asking about enabling factors and barriers to bereavement care provision. Nursing officers and registered and enrolled nurses working in ED of 2 urban acute general hospitals were recruited.

Results: Sixty-six questionnaires were returned out of 100 distributed. The mean age of the nurses was 31.4 (Standard deviation 8.7) years. Almost all the nurses (97%) felt that bereavement care should be provided by support services e.g. medical social services. Most nurses (77%) felt that bereavement care is an extension, rather than a natural part of their nursing role. The majority of nurses (92%) felt that both registered and enrolled nurses should play equal roles in providing bereavement support and that doctors should play an active role too. Lack of manpower is deemed the most important barrier while appropriate training and establishing a dedicated bereavement care service are considered the most important enabling factors in delivering bereavement care.

Conclusion: The majority of ED nurses do not perceive bereavement care as part of their natural nursing role. The ED needs to decide if ED nurses should provide bereavement care, and if so, change their perceptions and provide the necessary resources including training.

I104/NUR

A Process Improvement to Provide Analgesia Earlier to Patients in Pain

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Aim: Traditionally, parenteral pain-relief was administered after a patient had seen an ED doctor. A pre-implementation study showed that an average waiting time for analgesia was 74 minutes (95% CI 69-79). To offer and administer analgesia to patients presenting with pain secondary to musculoskeletal injuries and minor injuries in the earliest possible time.

Methods: A protocol-based nurse-delivery of analgesia was implemented on 18 April 2004. All patients presenting with pain secondary to musculoskeletal injuries and minor injuries were asked at triage to rate their pain intensity on the Numeric Rating Scale (NRS) or Faces Pain Scale with a range of 0-10. To

patients with NRS of equal or more than 7, the triage nurse offered intramuscular Ketorolac as per protocol. If NRS was less than 7, the nurse would give intramuscular Ketorolac upon request if there was no contraindication. We compared the mean door-to-analgesia time for month-long samples pre and post study implementation.

Results: Of the 554 patients presenting with pain secondary to musculoskeletal injuries and minor injuries, 96 patients were given analgesia at triage. Seventy-two patients had NRS of equal to or more than 7. Twenty-four patients had NRS of less than 7. Forty-six patients declined analgesia although their NRS was equal to or more than 7. The mean door-to-analgesia time was 13.2 minutes (95% CI, 11-15). The door-to-analgesia time was reduced by 82%.

Conclusion: A change in processes whereby a nurse-initiated pain management protocol effectively allowed earlier delivery of parenteral analgesia to patients in pain.

I105/NUR

Utility of Subjective Visual Impairment in Managing Frail Elderly Outpatients

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Aim: To examine the utility of a patient-response screening question for visual impairment with regards to diagnosis and impact on function (alone and in combination with hearing impairment).

Methods: Retrospective study of 124 consecutive new cases attending the geriatric assessment clinic, Tan Tock Seng Hospital, from June to December 2003. All cases underwent nurse clinician assessment before clinical consultation. The screening question for visual impairment was compared with objective measurement by Snellen's chart (>6/12 visual acuity in better eye); hearing impairment was determined by the whisper test.

Results: The prevalence rate of visual impairment was 81.3%. Only 2 patients (0.2%) had a vision-linked complaint, with none being the presenting complaint. The screening question had lower specificity (42.9%) than sensitivity (68.1%) for decreased visual acuity; this improved to 54.5% when compared with an alternate definition incorporating monocular visual impairment. It was able to identify those with falls ($P < 0.05$).

Conclusion: Visual impairment is common and can be easily missed unless systematically looked for. Subjective visual impairment appears to be able to identify a select group with monocular visual impairment and functional consequences. We propose the strategy of a patient-response screening question for visual impairment, combined with whisper test, for detection of significant functional impairment in management of frail elderly outpatients.

I106/NUR

A Study on the Utilisation of Dementia Day Care Services

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Aim: 1) To determine the acceptance of dementia day care (DDC) centre referrals by carers of newly diagnosed dementia patients. 2) To explore the reasons why some carers decline referrals to DDC.

Methods: This is a prospective, observational study involving carers of 100 consecutive cases of newly diagnosed dementia. Within the first 3 months of diagnosis, each carer was given standardised information on the benefits of DDC and the subsequent response to a suggested DDC referral was documented. The relationship between the carers' response and patient-, carer- and facility-related variables were explored.

Results: Of the 100 carers studied, 91 declined DDC referral, giving an acceptance rate of only 9.0%. The decision by caregivers to utilise DDC was significantly related to the stress level experienced, as measured by the Relative Stress Scale (RSS) ($P = 0.031$). Among these, fear of accidents to patients ranked as the most stressful item, followed by feelings of anger and frustration at patients. Among those who declined DDC referral, 61 carers (67.0%) felt that they were coping well, while 56 carers (61.5%) did so out

of agreement with patient's expressed rejection of DDC.

Conclusion: The decision by carers to utilise DDC is significantly associated with the stress level experienced. Conversely, carers who are coping well do not see the need for DDC despite being explained the possible cognitive and social benefits to patients. Many carers also tend to go along with the expressed refusal of DDC by patients.

I107/NUR

The Usage of Alternative/Complementary Medicine Among Parkinson's Disease Patients

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Aim: To determine the frequency of usage of alternative/complementary medicine (ACM) among patients with Parkinson's Disease (PD) in Singapore.

Methods: A structured questionnaire was administered by interview to 160 consecutive PD patients seen at the Movement Disorders Clinic of National Neuroscience Institute, Singapore, over an 8-week period about their use of ACM for PD. Patients who had concurrent dementia with CMMSE equal or less than 21 were excluded.

Results: A total of 98 patients used ACM for PD. The mean age of the patients was 60.8 years with 67 (68.3%) male patients. The ethnicity of these 98 patients was as follows: 85 (86.7%) Chinese, 8 (8.1%) Indians, and 5 (5.1%) Malays. Ninety-eight patients used 200 forms of ACM; of these, 35 (35.7%) patients used 1 ACM, 36 (36.7%) patients used 2 ACMs, 19 (19.3%) used 3 ACMs, 5 (5.1%) patients used 4 ACMs, and 3 (3.0%) patients used 5 ACMs. The 4 most commonly used ACM among patients were Traditional Chinese Medicine (TCM) (51%), acupuncture (44.9%), vitamins (37.7%), and massage therapy (31.6%). The least commonly used were Traditional Malay Medicine (TMM) and aromatherapy at 0.02% each. The majority (83.7%) did not inform their doctors about their use of ACM. Five patients (5.1%) missed their anti-PD medicines while on ACM. Of the 200 forms of ACM used, 120 (60%) did not improve the PD symptoms.

Conclusion: The use of ACM is common among PD patients. The majority did not inform their doctors about their use of it.

I108/NUR

Hand Hygiene Compliance by Healthcare Workers in an Acute Hospital

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Aim: Hand hygiene is critical in preventing cross-infections in hospitals. However, reports of hand hygiene compliance among healthcare workers (HCWs) have been low at 30% to 50%. The objective of this study was to evaluate the baseline hand hygiene compliance in Tan Tock Seng Hospital (TTSH) before and after patient contact.

Methods: An audit of hand hygiene was conducted in Tan Tock Seng Hospital in June 2004. Three hundred and thirty HCWs in 30 wards were observed during 2-hour periods of the morning and afternoon shifts for 2 weeks.

Results: Hand hygiene before and after patient contact was 3.6% and 53% respectively. HCWs were more likely to clean their hands after "long" patient contact (72.2%) than after "short" patient contact (30%). However, among those HCWs who practised some form of hand hygiene after patient contacts, 95.4% did not adhere to the recommended 6-steps handwashing technique. Allied HCWs had the highest compliance rate for hand hygiene after patient contact. Non-compliance to hand hygiene after gloves removal was highest among enrolled nurses/healthcare assistants (26%).

Conclusion: Hand hygiene is the most effective way to prevent nosocomial infections. Knowing the baseline rates of hand hygiene compliance is essential in planning active intervention programmes to improve hand hygiene practices. Understanding why different groups of HCW's seem to have different levels of compliance would help in designing different strategies to improve adherence to hand hygiene.

I109/NUR**Compliance Therapy in Psychotic Patients a Pilot Study on an Intervention to Improve Inpatients Attitude and Compliance with Treatment**SE TAY*Department of Nursing, Institute of Mental Health/Woodbridge Hospital, Singapore*

Aim: A brief intervention, compliance therapy based on motivational interviewing and cognitive approaches lead to improved attitudes to treatment (Kemp et al, 1996, 1998). During patient education session, the researcher who was trained in cognitive-behavioural therapy, and attended a workshop on motivational interviewing, practised the intervention as described. The shortened version of Drug Attitude Inventory (DAI-10) (Hogan et al, 1983; Dongen, 1997) had been used routinely as a measurement tool before and after patient education. This descriptive study aims to examine the effectiveness of using Compliance Therapy and ways of improvement.

Methods: Patients who were referred for patient education by the doctor and those who were put on the clinical pathway of relapsed schizophrenia/depression in the same ward were included as the sample subjects. A rating scale adapted from Kemp et al (1998) was used to rate patients' compliance.

Results: Among the 69 male subjects, their mean age was 43.5 years. Their mean length of illness was 16 years. The patients in individual or group session both had significantly improved attitude scores after the intervention. Patients with less than 5 previous admissions had more significant improvement but patients with personality disorder and/or history of substance abuse had no significant improvement.

Conclusion: Compliance therapy could be conducted in the form of small groups of 2 to 3 patients but patients with lower pre-DAI-10 score would benefit more in individual session.

I110/NUR**Coping Differences of Stress Between Male and Female Graduates in Hong Kong Australia and Singapore**NS LIEW*Department of Nursing, Institute of Mental Health/Woodbridge Hospital, Singapore*

Aim: Coping strategies function as a vital role in the individual's physical and psychological health when faced with negative life occurrences. With the changing work expectations many graduates choose to upgrade their skills. They had to juggle their time with family, study and work, thus, coping with stress. Hence, the aim of this study is to examine the coping differences of stress between the male and female graduates from Hong Kong, Singapore and Australia.

Methods: Two hundred ninety-nine graduates who read Master of Social Counselling responded to the Student-Life Stress Inventory and Coping Inventory for Stressful Situations. Their coping differences were examined as based on the 5 dimensions: task-orientation, emotion-orientation, avoidance-orientation, distraction and social diversion. Inferential statistics with 2 tailed t-test ($P < 0.05$) results were used

Results: The results indicated that the male and female graduates' coping strategies on task-orientation ($P = 0.113$) were insignificant. Though the mean score of task-orientated coping showed that the males ($M = 58.08$) was slightly higher than the females ($M = 56.08$). This finding is similar to Endler and Parker (1990; 1994). Moreover, the females use more emotion-orientation ($P = 0.026$), avoidance-orientation ($P = 0.002$), distraction ($P = 0.001$) and social diversion ($P = 0.033$) coping styles compared to the male graduates. These results confirmed the earlier researchers' findings (Endler & Parker, 1990; Higgins & Endler, 1995).

Conclusion: The finding on the coping differences of stress between male and female graduates on task-orientation was similar ($P = 0.113$). Moreover, the female graduates used more emotion-orientation than their male counterparts.

I111/NUR**Socio Demographic Profile and Outcomes of Relapsed Schizophrenic Patients on Clinical Pathway**MM HENDRIKS¹, R MAHENDRAN², V THAMBYRAJAH², SCB LIM¹,UJA KRISHNAN¹, YH CHAN³*¹Department of Nursing, Institute of Mental Health/Woodbridge Hospital, Singapore, ²Department of General Psychiatry, Institute of Mental Health/Woodbridge Hospital, Singapore, ³Research Division, Institute of Mental Health/Woodbridge Hospital, Singapore*

Aim: The clinical pathway for Relapsed Schizophrenia was introduced in Woodbridge Hospital in July 2003. There are 4 other clinical pathways in use, namely, Major Depression, First Episode Schizophrenia/Schizophrenic Disorder, Alcohol Dependence and Opiate Dependence. This study on 307 patients placed on the Relapsed Schizophrenia pathway from July 2003 to September 2003, aimed at capturing the socio demographic profile of the patients and the significant features that may contribute to their readmission.

Methods: A survey form was designed to capture the data. The researchers then audited the case notes of patients placed on the clinical pathway. Data was coded into the SPSS system and analysed.

Results: There were almost equal numbers of males and females with 42.3% experiencing the illness >10 years, 53.1% having at least 1 to 2 admissions during the year, 74.6% unemployed and 88.6% living with their families. Males were more likely to be readmitted ($P = 0.009$), as well as those with 10 years' duration of the illness ($P = 0.003$). Other significant predictors of readmission are those with at least 1 admission in the past year ($P = 0.025$) and those that did not come for their follow up after discharge ($P = 0.002$).

Conclusion: Clinical pathway assist healthcare in providing quality care for patients through its systemised structure of activities. It also enables the practitioners to collect data on their practices to improve their services to their patients.

I112/SC**Risk Scoring Systems for Preoperative Renal Dysfunction Asian Patients Who Undergoing Open Surgery Using Cardiopulmonary Bypass**GX XU¹, HD LUO², MD COSTA¹, PS WONG¹, CTT TAN¹, EKW SIM¹, CN LEE¹*¹Department of Cardiac, Thoracic and Vascular Surgery, National University Hospital, Singapore, ²Department of Medicine, National University Hospital, Singapore*

Aim: Risk scores are useful for comparing outcomes of open-heart surgery using cardiopulmonary bypass. Different scoring systems are based on different designs and patient populations. This study aims to evaluate the applicability of the Euroscore and Northern New England system to preoperative renal dysfunction Asian patients who undergoing open-heart surgery using cardiopulmonary bypass.

Methods: From 1 September 2002 to 31 October 2003, data of 121 consecutive preoperative renal dysfunction Asian patients who undergoing open-heart surgery using cardiopulmonary bypass in National University Hospital (Singapore) was prospectively collected. Euroscore and Northern New England systems were used to evaluate the mortality predictions for all subjects. Validity of these risk scoring systems was assessed by the area under the receiver operating characteristic (ROC) curve.

Results: The observed mortality for preoperative renal dysfunction Asian patients who undergoing open-heart surgery using cardiopulmonary bypass was 10.7% in this study. The predicted mortalities were $8.7 \pm 4.2\%$ by Euroscore, and $5.7 \pm 4.5\%$ by the Northern New England scoring system. The areas under the ROC curves were 0.77 ($P = 0.01$) for Euroscore and 0.7 ($P = 0.08$) for Northern New England scoring system.

Conclusion: Although derived from a predominantly Caucasian population, Euroscore still can be applicable to preoperative renal dysfunction Asian patients who undergoing open-heart surgery using cardiopulmonary bypass.

I113/SC**Tracheobronchial Airway Stenting for Relieving Malignant Airway Obstruction**PS WONG¹, CF CHONG², HL LIM³, KL KHOO⁴, A CHANG⁵*¹Department of Cardiac, Thoracic and Vascular Surgery, National University Hospital, Singapore, ²Department of Cardiac, Thoracic and Vascular Surgery, National University Hospital, Malaysia, ³Department of Haematology-Oncology, National University Hospital, Singapore,*

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Aim: Obstruction of the airways due to malignant disease is a frightening condition that portends a poor prognosis. Emergency airway stenting can quickly palliate and relieved the obstruction. We retrospectively analysed our data on the management of these patients.

Methods: From 1999 to 2004, records of 11 patients with urgent tracheobronchial stenting using rigid bronchoscopy for palliative relief of airway obstruction were reviewed.

Results: The median age of these 11 patients (6 males and 5 females) was 43 (range, 18 to 69) years. The diagnoses were as follows: NSCLC (7), adenoid cystic carcinoma (1), small cell lung cancer (1), Hodgkin's lymphoma (1) and malignant thymoma (1). A total of 15 airway stents were inserted consisting of 2 tracheal stents, 7 left bronchial stents and 6 right bronchial stents (6 covered/5 uncovered ultraflex stents and 4 polyflex stents). All patients had immediate symptomatic relief. Follow-up is as follows: 6 patients died within a year of stent insertion from their underlying malignancy, 2 were transferred back to their respective countries and were lost to follow up, the 3 remaining patients are still being follow-up by the oncologist. The patient with Hodgkin's lymphoma had regression of her disease with chemotherapy and is well 2.5 years after stent placement.

Conclusion: Malignant airway obstruction from extrinsic or intrinsic causes can be managed by urgent palliative airway stenting, which provide instant relief in an otherwise fatal condition. Their outcome, however, is still poor and is determined by their underlying malignant disease.

I114/SC

Clinical Thyroid Pathways Improve Healthcare for Patients Undergoing Thyroidectomy

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Aim: Cost containment and delivery of cost-effective surgical care have become important considerations for hospitals. Clinical Pathways is one such tool that has been developed to address these problems. In this study we evaluated the effect on cost and clinical outcome with the implementation of clinical care pathway for thyroidectomy patients.

Methods: The thyroidectomy pathway was implemented in 2001, through collaborative efforts of clinicians, case managers, and nurses. From 2002 to 2003, 143 patients were managed on clinical pathway compared to 150 patients (2000 to 2001), who served as controls. All patients were managed in the Department of Surgery.

Results: Control group; age range 15 to 88 (mean, 48.2) years (55 total thyroidectomy, 95 hemithyroidectomy cases). Pathway group; aged between 10 and 83 (mean, 44.4) years (45 total thyroidectomy, 98 hemithyroidectomy cases). Age, co-morbidity, complications, readmission rates did not show much difference between the two groups ($P > 0.05$). There were no deaths for both groups. Mean total length of stay was less in the postclinical pathway patients (1.91 days) compared to preclinical pathway patients (3.33 days) ($P < 0.001$). Mean hospital charges also decreased significantly, from \$3,929 to \$3,524 per patient ($P = 0.003$).

Conclusion: Clinical pathways have been shown to reduce unnecessary variation in patient care, reduce delays in discharge, and improve cost-effectiveness of clinical services. Our study confirms that length of stay and hospital costs are effectively reduced through the implementation of clinical pathway for patients undergoing thyroidectomy.

I115/SC

Multidetector CT in Acute Lower Gastrointestinal Haemorrhage

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Aim: To evaluate the accuracy of multidetector CT (MDCT) in the evaluation of patients presenting with acute lower gastrointestinal bleeding. Acute lower gastrointestinal bleeding is a potentially life-threatening condition which has

historically been evaluated with catheter angiography if endoscopy fails to reach a diagnosis or is incomplete or unsuccessful. Unfortunately, angiography is both invasive and has a very low sensitivity both in our institution and in reports published in the literature. Recently there have been some reports on the use of MDCT in the evaluation of these patients. We set out to validate this technique in our local patient population.

Methods: Twelve consecutive patients presenting with acute lower gastrointestinal bleeding with incomplete, failed or nondiagnostic endoscopy were referred for contrast enhanced MDCT. These patients subsequently had repeat endoscopy or surgery.

Results: MDCT scan was technically successful in all patients, showed the site of bleeding in all patients, and suggested cause in some patients. The patients underwent subsequent repeat endoscopy or surgery to treat the bleeding and confirm cause of bleeding. The detailed radiological, endoscopic, surgical and final histological diagnosis will be presented.

Conclusion: MDCT is a useful, sensitive and accurate technique for evaluating patients with acute lower gastrointestinal bleeding.

I116/SC

Imaging Soft Tissue Tumours

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Aim: There is a wide range of modalities available for assessment of the soft tissues. This study aims to illustrate how different modalities contribute to the final diagnosis of a soft tissue mass.

Methods: Consecutive cases of soft tissue masses of the upper limb which underwent higher modality imaging in the Diagnostic Imaging Department of National University of Singapore from January 2001 to March 2004 were retrospectively reviewed. Of these, 64 cases had histological correlation. We present selected cases which best illustrate the contribution of each modality to the final diagnosis.

Results: There were 37 cases of benign tumour, 7 cases of malignancy, 15 cases of inflammation and 5 cases of reactive change (reactive lymph nodes or fibrosis).

Conclusion: We suggest plain film should be used for initial evaluation of these masses in order to determine the presence of calcification or fat and to demonstrate any associated bone involvement. In many cases ultrasound will confirm the presence of a benign cystic lesion, which may require no further imaging. MRI however remains the modality of choice for many soft tissue lesions in order to determine the origin and extent of the mass. The MR signal characteristics of the lesion may also be helpful in determining the final diagnosis.

I117/SC

Missed Osteosarcoma

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Aim: Several patients with extremity osteosarcomas have been treated by one of the authors (Pho RWH) over the years where the diagnosis of osteosarcoma was not made at initial presentation. A review was conducted to find any signs that could have alerted the clinician to this diagnosis at an earlier stage.

Methods: We conducted a retrospective review of 6 patients with extremity osteosarcomas where the diagnosis was initially missed, analysing how and why the diagnosis was missed at initial presentation.

Results: All the cases had a history of trauma and the attending doctors were unaware of an underlying pre-existing early malignant condition. The radiological findings were subtle and the patient's general condition at initial presentation was normal.

Conclusion: Since extremity osteosarcoma is uncommon, we emphasise a high index of suspicion of malignancy if the natural progression of the original diagnosis of trauma is not seen or if the signs and symptoms worsen. The outcome was significantly poor in all the cases because of a delay in diagnosis.

I118/SC

Cerebral Autoregulation and Pressure Reactivity Following Decompressive CraniectomyE WANG¹, BT ANG¹, J LIM², I NG¹¹Department of Neurosurgery, National Neuroscience Institute, Singapore,²Clinical Trial Epidemiology Research Unit, Singapore

Aim: Analysis of slow waves in arterial blood pressure (ABP) and intracranial pressure (ICP) has been used as an index to describe cerebrovascular pressure-reactivity. It has been previously demonstrated that the pressure-reactivity index (PRx) can be used to reflect global cerebrovascular reactivity with changes in ABP. A positive PRx signifies a positive association between ABP and ICP, indicating a non-reactive vascular bed while a negative PRx is reflective of intact cerebral autoregulation, where ABP waves provoke inversely correlated waves in ICP. To date, there has been no characterisation of pressure-reactivity following decompressive craniectomy.

Methods: In this prospective observational study, 33 patients who underwent decompressive craniectomy for raised intracranial pressure were studied. The PRx was calculated as a moving correlation coefficient between 30 consecutive samples of values of ICP and ABP averaged for a period of 10 seconds. The time profiles of PRx at 6-hourly intervals were then analysed.

Results: In all patients, the initial PRx 6 hours after surgery was positive, indicative of disturbed pressure-reactivity. With time, the PRx trended towards a more negative value, suggestive of an improving cerebrovascular autoregulatory reserve. The mean PRx at 24 hours was 0.3520 while the mean PRx at 72 hours was 0.2047 ($P < 0.001$).

Conclusion: Surgical decompression, apart from playing a crucial role in management of raised intracranial pressure may have a contribution to the restoration of disturbed cerebrovascular pressure-reactivity.

I119/SC

Changes in Cerebral Haemodynamics and Chemistry During Surgical Evacuation for Hypertensive Intracerebral Putaminal HaemorrhageIHB NG¹, E YAP², J LIM³¹Department of Neurosurgery, National Neuroscience Institute, Singapore,²Department of Research, National Neuroscience Institute, Singapore,³Clinical Trial Epidemiology Research Unit, Singapore

Aim: The efficacy of surgical decompression in spontaneous intracerebral haematoma in improving outcome is still controversial. Ischemic cell death in the penumbra is likely to be caused in part by intracranial hypertension and decreased cerebral perfusion pressure. Nevertheless, the effects of surgical decompression on neurological parameters have not been systematically studied. The aim of this study was to evaluate the changes in ICP, CPP, PtiO₂, PtiCO₂, brain pH and regional cerebral blood flow (rCBF) by laser doppler flowmetry (LDF) before and after surgery for spontaneous intracerebral haematomas.

Methods: Eight patients who underwent surgical decompression of spontaneous putaminal haematoma were studied. ICP, CPP, PtiO₂, PtiCO₂, brain pH and regional CBF (LDF) was recorded prior to removing the bone flap and then on skin closure on completion of the operation.

Results: Following surgical decompression, mean ICP decreased from 30.5 ± 11.77 to 12.27 ± 9.84 mm Hg ($P < 0.05$); brain tissue oxygenation improved from 18.11 ± 20.01 to 29.75 ± 27.74 mm Hg ($P < 0.05$); brain tissue carbon dioxide changed from 44.75 ± 11.29 to 45.88 ± 15.97 mm Hg ($P < 0.05$); brain pH improved from 6.87 ± 0.34 to 7.09 ± 3.53 ($P < 0.05$). LDF changed from 10.01 ± 6.26 to 11.45 ± 17.50 mL/min/100 g brain ($P < 0.05$).

Conclusion: Surgical decompression for spontaneous intracerebral haematomas does lead to significant reductions in ICP. Improvements in CPP, PtiO₂ and rCBF in the penumbra accompany this to a lower extent.

I120/SC

Invasive Multimodality Neuro-Monitoring in Neurosurgical ICU

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Aim: The pathophysiology of severe brain injury resulting from various traumatic and non traumatic causes is complex. Continuous invasive

neuromonitoring techniques measure different variables such as brain temperature (BT), brain tissue (PtiO₂) oxygenation, intracranial pressure (ICP) etc. The purpose of the study was to analyse the utility of this online information to compare parameters which predict mortality.

Methods: Twenty-four patients were admitted to the NICU, underwent invasive neuromonitoring, were intubated, sedated and ventilated as per the NICU protocol (GCS <9). BT, ICP, PtiO₂ probes were inserted as an initial procedure or combined with the surgical decompression. The MAP, RT, PtiO₂, ICP, CPP, BT were continuously measured and recorded.

Results: The mean age was 43.8 years. The diagnoses were: closed head injury in 15, basal ganglia bleed in 7, gunshot in 1 and AVM bleed (arteriovenous malformation) in 1. There were 20 survivors. The mean first 24 hours CPP (cerebral perfusion pressure) was lower in nonsurvivors at 60 mmHg as compared to survivors at 80 mmHg ($P < 0.05$). The groups total mean brain temperature fell below the mean total rectal one in non survivors (TOTBT = 34.24°C vs TOTRT = 34.41°C) suggestive of loss of temperature regulation due to the ensuing brain death process. Survivors maintained a positive temperature gradient (TOTBT = 37.61°C , TOTRT = 36.45°C). The survivors also demonstrated a higher TOTPTiO₂ (16.3 mmHg vs 2.8 mmHg in nonsurvivors) ($P < 0.05$). There were no complications related to the insertion of the multimodality monitoring device.

Conclusion: BT, PtiO₂ and CPP were found to be important factors independently influencing outcome. Invasive multimodality neurointensive monitoring can help in directing therapy to improving these parameters and prognosticate outcome in patients with severe brain injury.

I121/SC

What is the Ideal Cerebral Perfusion Pressure in Severe Head Injury (SHI)? – A Multivariate Logistic Regression StudyKK LEE¹, HB WONG², IHB NG³¹Department of Nursing, National Neuroscience Institute, Singapore,²Clinical Trial Epidemiology Research Unit, Singapore, ³Department of Neurosurgery, National Neuroscience Institute, Singapore

Aim: Maintenance of adequate CPP is important in SHI to minimise secondary insults from cerebral ischaemia. Conventional methods recommend CPP > 70 mmHg, but some studies advocate lower CPP to minimise risks of medical complications inherent in maintaining a high perfusion pressure. As there is more evidence towards range of 60 to 70 mmHg, the objective of this study was to relate total length of time CPP fall below threshold 60, 65, and 70 mmHg to outcome at 6 months.

Methods: All SHI patients GCS < 8 on ABP and ICP monitoring are included in this prospective clinical study. Statistical analysis was based on multivariate logistic regression for survival/mortality. The analysis strategy was to first fit a base model regressing outcome on age, gender, GCS and pupil abnormality. The total length of time CPP fall below threshold 60, 65, and 70 mmHg were added to the model to explore the extent to which they improved the predictive ability beyond the base model.

Results: 28 male and 9 female (mean age 44 years) were included in the study. Thirteen had pupillary abnormality. At 6-months follow-up, 27 are alive and 10 dead. By comparing the area under ROC curve for the 3 models, the model with cut-off total length of time CPP < 65 is a better model (highest ROC values = 0.9148) to predict the outcome (GOS = dead).

Conclusion: After statistical control of age, gender, GCS, pupil abnormality, total length of time CPP < 65 predict a worse outcome at 6-months.

I122/SC

The Role of Androgen Therapy in Female Sexual Dysfunction in the Menopause – A Systematic Review

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Aim: Female sexual dysfunction (FSD) is a common condition affecting postmenopausal women. We reviewed all randomised trials of androgen therapy for sexual function in menopausal women, looking particularly at estrogen-progesterone-androgen, estrogen-androgen, and androgen alone.

Methods: A literature search was done to identify all relevant RCTs. Sources

included MEDLINE, Cochrane Central database and references of published articles. Search terms included estrogen, androgen, menopause, sex behaviour, randomised controlled trial. We found 25 studies involving 20,153 women. Meta-analysis was performed where possible.

Results: There were 7 randomised trials (566 women) comparing estrogen-androgen therapy with estrogen replacement alone and with placebo. Androgen used alone or with ERT increased interest in sex (SMD 0.39, 95% CI 0.10, 0.69), frequency of orgasm (SMD 0.60, 95% CI -0.10, 1.30), sexual satisfaction (SMD 0.16, 95% CI -0.13, 0.46), sexual responsiveness (SMD 0.25, 95% CI -0.05, 0.55). When compared with ERT alone, concomitant use of androgen further improved interest in sex (SMD 0.23, 95% CI 0.01, 0.46), sexual responsiveness (SMD 0.32, 95% CI 0.09, 0.55), frequency of orgasm (SMD 0.24, 95% CI -0.12, 0.60) and general sexual satisfaction (SMD 0.23, 95% CI -0.19, 0.66), while reducing frequency of dyspareunia (SMD -0.34, 95% CI -0.77, 0.08). Androgens did not significantly increase total, HDL or LDL cholesterol levels when compared with placebo.

Conclusion: Evidence suggests that androgen therapy improves sexual function in the menopause, especially when administered with ERT. Lipid profile was not adversely affected. Androgen therapy may be considered for use in menopausal women with sexual dysfunction.

1123/SC

Efficacy of Intravitreal Triamcinolone Injections for Refractory Macular Oedema – Preliminary Results

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Aim: To evaluate the effect of intravitreal triamcinolone acetonide in patients with refractory macular oedema and to assess the clinical side effects of treatment.

Methods: Nine patients (nine eyes) with refractory vasculopathic macular edema associated with diabetes mellitus (n = 6) and central retinal vein occlusion (n = 3) were treated with intravitreal triamcinolone acetonide and followed up for at least 3 months.

Results: Significant reduction in the central macula thickness could be detected both clinically and on Optical Coherence Tomography as early as 1 week post treatment. No significant complications occurred in these 9 injections.

Conclusion: Intravitreal triamcinolone acetonide is safe and well tolerated. Preliminary data showed promising results in clinical and anatomical resolution of macular oedema.

1124/SC

Diabetic Retinopathy Study

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Aim: To determine the degree of severity of diabetic retinopathy at first visit of patients with diabetes mellitus and to study their knowledge, health beliefs and medical risk factors.

Methods: A prospective multi-centred cohort study. The inclusion criterion is every consecutive patient with a medical history of diabetes mellitus seen in eye clinics of 6 public hospitals at first visit or who have defaulted from eye consultation for 1 year or more. Each patient will be interviewed by a trained research assistant. The degree of diabetic retinopathy will be assessed by ophthalmologists and staged according to the criteria from the Early Treatment Diabetic Retinopathy Study.

Results: A total of 923 patients were interviewed. The majority of them (79%) were between 41 and 70 years of age. Eighty-five per cent of all subjects had none or mild non-proliferative diabetic retinopathy and 8% had either severe or proliferative diabetic retinopathy. Only 47% of the entire cohort had heard of diabetic retinopathy and 52% knew the significance of yearly eye screening. Ninety-one per cent of them were non-alcohol drinkers

and 86% were non-smokers. Hypertension and hyperlipidaemia were present in 45% and 32% of them respectively and 11% had underlying heart disease. Only 61% had yearly diabetic photographic eye screening. Thirty-five per cent of the patients screened needed laser treatment and 3% required vitrectomy and laser therapy.

Conclusion: A significant number of patients still presented late on first eye assessment requiring laser or surgical treatment. Diabetic patients need to be better educated with the condition as well as the importance of diabetic eye screening.

1125/SC

Knowledge, Attitude and Perceptions of Current Smokers Attending an Ophthalmology Outpatient Clinic Towards Smoking-Related Diseases – A Preliminary Report

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Aim: Smoking is a modern day epidemic that confers risks of serious illnesses, and its cessation offers health benefits. This study seeks to evaluate the knowledge, awareness and fear of smoking-related diseases among current smokers attending an ophthalmology outpatient clinic to understand their motivation to quit smoking.

Methods: Following a complete eye examination, ophthalmic outpatients who were current smokers were interviewed using a standardised interviewer-administered questionnaire on their knowledge and awareness of smoking-related diseases.

Results: This preliminary report comprised 34 current smokers with a mean age of 66.6 (range, 55 to 80) years. There were 33 male and 1 female smokers. 61.8% were retirees. The visual acuity ranged from 6/6 to no light perception. The proportions of smokers able to attribute specific conditions to smoking were lung cancer (73.5%), other lung diseases (64.7%), heart attack (61.8%), stroke (52.9%), other cancers (50%), and blindness secondary to age-related macular degeneration (41.2%). 38.2% of smokers felt that they would like to prevent and 41.2% felt that they would like to treat heart attack first if it were possible to prevent or treat only one. The same proportion of respondents (17.6%) preferred to treat blindness and lung cancer first.

Conclusion: While most smokers recognise many health hazards of smoking, their awareness of blindness is low. Heart attack is a far greater source of concern than any other illness. Public education efforts to increase the awareness of blindness as a smoking-related disease could potentially help in motivating smokers to quit smoking in antismoking campaigns

1126/SC

Central Corneal Thickness in Singapore Children

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Aim: Little is known about central corneal thickness (CCT) in children. The establishment of normal CCT values in children is important because this aspect of ocular growth is currently not well understood. It may affect interpretation of estimated intraocular pressure (IOP), our understanding of ocular refractive error development and the mechanical properties of the cornea. We aimed to establish the variation of CCT among normal Singapore children.

Methods: Cross-sectional results of 652 right eyes of children aged 9-11 years in the Singapore Cohort Study of the Risk Factors for Myopia (SCORM) were evaluated in a government primary school. CCT was measured using OLCR slit-lamp pachymeter and IOP using non-contact air puff tonometer. Post-cycloplegic refraction and corneal curvature radii were measured using auto-refractometer.

Results: Mean CCT of Singapore children aged 9-11 years was 543.6 μm . The Chinese had thicker mean CCT (546.0 μm) compared to the Malays (539.5 μm) and Indians (532.3 μm) ($P = 0.002$). Boys had thicker CCT ($P = 0.011$). Higher IOP correlated with thicker CCT (Pearson $r = 0.45$, $P < 0.001$). Larger corneal curvature radii weakly correlated with thicker CCT ($P < 0.001$). No relationship was observed between CCT and age, refractive error status and axial length of eyes.

Conclusion: Mean CCT values in Singapore children did not differ greatly from that reported in Singapore adults in the Tanjong Pagar Study. Our results are surprising as another study in Hong Kong Chinese showed a main decline in CCT between age groups 5-15 and 15-25 years. It may be necessary to consider CCT variations when obtaining IOP estimates in children.

I127/SC

Paediatric Epiphora – Is It All Due to Nasolacrimal Duct Obstruction?

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Aim: 1) To identify etiology of epiphora in infancy and childhood 2) To assess efficacy of probing 3) To identify clinical entities presenting as Nasolacrimal Duct Obstruction (NLDO).

Methods: Case notes of 20 children (29 eyes) with epiphora who underwent surgical intervention between April 2000 and April 2004, at Eye Clinic, National University Hospital were retrospectively analysed. The children were in the age group 11 months to 6½ years at intervention. The surgeries were performed by 2 Ophthalmic surgeons trained in Oculoplastics. Glaucoma and surface/lid infection were excluded.

Results: Of 29 eyes, 58.62% ($n = 17$) were watering due solely to typical NLDO. 41.38% ($n = 12$) eyes had other associations causing / contributing. These were: Epiblepharon, Punctal atresia, Common canalicular block, inferior turbinate hypertrophy. 68.95% ($n = 20$) eyes of 15 children warranted Probing alone. Of these, 40% ($n = 8$) were totally relieved. Another 40% ($n = 8$) were partly relieved. Ten per cent ($n = 2$) had persistence of symptoms. Ten per cent were ($n = 2$) lost to follow-up. Additional procedures that were indicated/performed included: Epiblepharon correction - $n = 7$ (24.13%), Infraction of turbinate - $n = 5$ (17.24%), Intubation - $n = 3$ (10.34%), punctoplasty - $n = 2$ (6.89%).

Conclusion: Less than 60% were due entirely to typical NLDO and not even half of Probing procedures were successful in totally relieving the symptoms. So, NLDO should not be presumed as the cause of Epiphora and Nasolacrimal Probe is no magic wand that cures the problem. If a reasonable period of conservative management does not relieve symptoms, Ophthalmic referral is worthy of (NLDO: nasolacrimal duct obstruction).

I128/SC

The Effect of Storage on the Efficacy of Reconstituted Dysport

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Aim: Botulinum toxin type A (BTX-A) (Dysport, Ipsen) labelling recommends its immediate use after reconstitution. As Dysport is available in 500-unit vials, a substantial quantity of this expensive drug is often discarded. With reference to 7 patients with forehead wrinkles, we compare the effect of freshly reconstituted Dysport with Dysport that had been refrigerated (+40°C) for 1 week on the action of frontalis.

Methods: Seven patients with symmetrical forehead wrinkles and who previously responded to Dysport injection of the frontalis were entered into the study. One side was injected with freshly constituted and the other side with reconstituted Dysport that had been stored for 1 week at +40°C. For each subject, an identical amount of Dysport was given to each side (20-40 units to each side). All injections were performed by the same ophthalmologist. The patients were reviewed at 12 weeks and were observed by a blinded independent observer for the excursion of the eyebrows using a ruler (which reflects the strength of the frontalis).

Results: The difference in the amount of eyebrow movement between the 2

sides was not statistically significant ($P < 0.05$). Importantly, there was no adverse effects such as infection from the injections.

Conclusion: Reconstituted Dysport that is subsequently refrigerated for 1 week does not lose its clinical efficacy when used in the treatment of forehead wrinkles.

I129/SC

Lid Contour Abnormality in Levator Advancement and the Location of the Tarsal Plate

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Aim: Lid contour abnormality in the form of peaking is a common cause of dissatisfaction following ptosis surgery. In this study, we look at the effect of the location of tarsal plate suture on peaking.

Methods: In this prospective study, 22 patients undergoing levator advancement are recruited. 11 patients had the tarsal plate suture placed at 4 mm from the superior tarsal border and 11 patients had the tarsal plate suture placed at 6 mm from the superior tarsal border. Any preoperative peaking was recorded and the sutures were then re-sited until no peaking is observed.

Results: Of the 11 patient with tarsal plate suture placed 4 mm from the superior tarsal border, 1 patient had peaking of the lid margin. The peakings were corrected when the sutures were placed nearer the superior tarsal border. Of the 11 patients with tarsal plate suture placed 6 mm from the superior tarsal border, 6 patients had peaking of the lid margin which resolved when the sutures were placed nearer the superior tarsal border. The results were statistically significant.

Conclusion: Peaking of the eyelid is related to the location of the tarsal plate suture. An anteriorly placed suture may lead to an uneven distribution of the force causing peaking. Peaking is less common with suture placed nearer the superior tarsal border.

I130/SC

Visual Experience During Ophthalmic Laser Procedures

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Aim: To describe patients' visual experiences during ophthalmic laser procedures and to determine if they found the visual sensations frightening.

Methods: A post-surgical questionnaire survey was conducted on patients undergoing ophthalmic laser procedures at The Eye Institutes at Tan Tock Seng and Alexandra Hospitals.

Results: One hundred and sixty patients with a mean age of 60.2 years were interviewed. Ninety-one were male (56.9%) and 69 were female (43.1%). There were 126 Chinese (78.8%), 23 Malays (14.4%), 9 Indians (5.6%) and 2 (1.3%) from other ethnic groups. The laser procedures performed were panretinal photocoagulation (50.9%), focal laser (31.9%), grid laser (3.8%), laser retinopexy (3.1%), Nd:YAG posterior capsulotomy (6.9%) and laser peripheral iridotomy (3.1%). Most of the patients experienced light perception during the procedure (98.1%). In addition, patients also experienced a variety of visual sensations such as colours (83.5%), flashes (75%), movement (51.3%), branching patterns of blood vessels (16.3%), the surgeon's hand (14.4%), the surgeon (6.9%), floaters (4.4%) and instruments (3.8%) with their operated eye during the procedure. Nineteen patients (11.9%) found their visual experiences frightening.

Conclusion: The majority of patients undergoing ophthalmic laser procedures experienced at least light perception. The visual experiences were frightening to 11.9% of patients. Counselling patients about the possibility of visual experiences when obtaining informed consent for ophthalmic laser procedures may help alleviate this fear.

I131/SC

A National Cytomegalovirus Retinitis Screening Programme: Evaluation of Enrolment Criteria

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Aim: Our study aimed to assess the pick-up rates of parameters which are used as an indication to initiate a screen for cytomegalovirus (CMV) retinitis in Singapore, to find the optimal combination that maintains a high pick-up rate and reduces the number of screenings required.

Methods: During a 12-month period from April 2001 to March 2002, all HIV patients with any of the following criteria: 1) Visual symptoms, 2) CD4 count <50 cells per microlitre, 3) AIDS defining diseases (ADD) or 4) CMV affecting other parts of the body (CMV elsewhere) were screened for CMV retinitis with both the binocular indirect ophthalmoscope and 9-field digital fundus photography. Various combinations of the above 4 criteria were assessed for their relative importance as enrolment criteria.

Results: During the study period, 153 HIV-positive patients were screened (341 screening visits). Of these, 14 (9.2%) had CMV retinitis. Using a single criteria, the pick-up rate ranged from 0% to 71.4%. With various combinations of 2 of the 4 screening criteria, the pick-up rate ranged from 14.3% to 85.7%. When 3 screening criteria were used, the pick-up rate ranged from 28.6% to 100%. A combination of visual symptoms, CD4 and ADD reduced workload by 5.3% while maintaining 100% pick-up rate.

Conclusion: Using a combination of visual complaints, CD4 count and ADD as criteria for screening, 100% of patients with CMV retinitis can be detected in this study population, while reducing the workload by about 5%.

I132/SC

Comparing Lateral Tarsal Strip Versus Full Thickness Pentagonal Resection In the Management of Involutional Ectropion

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Aim: To compare the results of lateral tarsal strip procedure with full thickness pentagon lid resection in the correction of involutional ectropion.

Methods: A retrospective study of 102 patients who underwent surgery for senile ectropion of lower lid from March 1995 to March 2001. All the patients underwent either lateral tarsal strip operation or full thickness pentagon resection. The following preoperative signs were recorded: site of ectropion, lid laxity and secondary lid margin changes. Success was defined as relief of symptoms and good lid position.

Results: Sixty-nine per cent (70/102) of patients had lateral tarsal strip and 31% (32/102) of patients had full thickness pentagon resection. Overall success was achieved in 90% (63/70) of patients who underwent lateral strip operation and in 90.65% (29/32) of patients with full thickness pentagon resection. However, in patients with medial ectropion (punctal and medial) the success rate was 88.46% (46/52) in lateral tarsal strip procedure compared to 95.65% (22/23) with full thickness pentagon lid resection ($P < 0.05$). In patients with secondary lid margin changes the success rate was 73.91% (17/23) in lateral tarsal strip procedure compared to 86.66% (13/15) with full thickness pentagon resection ($P < 0.05$).

Conclusion: Both lateral tarsal strip and pentagon resection are effective in the treatment of involutional lower lid ectropion. However, in patients with medial ectropion and those with secondary lid margin changes, full thickness pentagon resection gives a higher success rate than lateral tarsal strip operation.

I133/SC

Management of Epiphora with Botulinum Toxin A

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Aim: Epiphora secondary to outflow obstruction is best treated surgically. However, not all patients are suitable for surgical intervention. The study looked at the effectiveness of lacrimal gland injection with Botulinum Toxin A as an alternative to surgery in controlling symptomatic epiphora.

Methods: Ten patients who had epiphora secondary to outflow obstruction were recruited for the study. All patients declined surgical intervention but found the epiphora troublesome. Informed consent was obtained for Botulinum Toxin A injection into the lacrimal gland. All patients had baseline measurements including Schirmer's test and marginal reflex distance

measurement. All received 10 units of Botulinum Toxin A injected transconjunctivally into the lacrimal gland.

Results: All patients reported a subjective improvement in their symptoms. Two patients suffered ptosis. One patient declined further injection. Eight patients experienced no side effects and found the treatment acceptable

Conclusion: Botulinum Toxin A injection into the lacrimal gland can be used as an alternative to surgery in patients with epiphora secondary to outflow obstruction.

I134/SC

Reconstruction of Large Lower Eyelid Defect with Mid Lift and Hughes' Tarso Flap

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Aim: The reconstruction of large lower eyelid full-thickness defect poses a challenge. The Hughes' procedure (tarso-conjunctival flap) has been used as a staged reconstructive surgery for the posterior lamella; with the anterior lamella often being reconstructed with full-thickness skin graft.

Methods: We describe the use of mid-face lift as an alternative in anterior lamellar reconstruction. A patient with squamous cell carcinoma of the lower eyelid had an 80% full-thickness defect after resection with frozen section monitoring.

Results: The posterior lamella reconstruction is achieved using the Hughes' procedure, while the anterior lamella, because of the huge skin defect, was managed with a mid-face lift to achieve the desired amount of flap advancement. The tissues over the temple and cheek are reliable sources for flap reconstruction of the lower eyelid. This technique proves to be ideal with the advantages of good skin colour match, excellent blood supply and survival (as compared to free skin grafts) and avoiding skin graft harvesting with donor site morbidity. The remaining orbicularis oculi in the midface enables the reconstructed lower eyelid to retain some function and close, in addition to the excellent aesthetic outcome.

Conclusion: The reconstruction of large full-thickness defects of the lower eyelid after tumor resection poses a challenging problem. This technique adds to armamentarium of surgical options for the reconstruction of large lower eyelid defects.

I135/SC

Measurement of Compartmental Pressures in Gluteal Compartment Syndrome

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Aim: Gluteal compartment syndromes are rare but potentially debilitating. The proximity of the sciatic nerve to the gluteal compartment makes it liable to injury. Thus early recognition and management are essential. There are a few case reports of this in literature but methods to measure pressures in the different gluteal compartments have not been described.

Methods: The anatomy of the gluteal compartments were studied in cadaveric specimens. Using an injectable colour dye, the compartments were distended and their extent studied. Using as control 1 side, the other buttock of the cadaveric specimen was used to inject dye into the various compartments and study them. The exact position and depth of needle placement for measurement of pressures in each of the gluteal compartments was recorded.

Results: The gluteal region consists of 3 well-defined compartments – the tensor fascia lata, the gluteus medius and minimus and the gluteus maximus. Each of these compartments are enclosed in a nondistensible osseofascial sheath. The sciatic nerve lies in between the gluteus medius and minimus compartment and short external rotators of the hip. Measuring the compartmental pressures in each of these compartments needs precise needle placement. The correct placement of the transducer needle used for measuring compartmental pressures for the 3 compartments will be discussed. A case presenting with bilateral gluteal compartment syndrome will also be discussed.

Conclusion: Recognition of gluteal compartment syndrome, methods of measurement of pressures in the gluteal compartment, relevant anatomy and the management options will be presented.

I136/SC

Is Computer Total Knee Arthroplasty More Accurate Than Standard Instrumentation Results of a Prospective Randomised Trial?

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Aim: Computer navigation has been advocated in total knee arthroplasty (TKA) to improve the overall accuracy and consistency of the postoperative limb alignment and positioning of the prosthesis. We conducted a prospective randomised controlled trial to verify this.

Methods: Ninety patients requiring TKA were randomised into 3 groups to compare the results between 1) intra-medullary femoral and tibial instrumentation (IM group), 2) intra-medullary femoral but extra-medullary tibial instrumentation (EM) and 3) Computer assisted navigated system (CA). Both clinical and radiological assessments including long leg films were made by a single independent reviewer and results compared with ANOVA test.

Results: The mean hip-knee-ankle angle is 3.1° varus for the IM group, 2.7° varus for EM and 1.5° varus for CA ($P = 0.013$). Both femoral and tibial alignment is also significantly better ($P = 0.004$). Similar findings are found when comparing femoral component flexion and posterior slope of the tibial component. The total postoperative drainage is also significantly lower in CA group ($P = 0.046$). The drop in haemoglobin level is also the least in the CA but this is not statistically significant ($P = 0.17$). However, the tourniquet time is 28 minutes longer in the CA group.

Conclusion: Computer assisted navigation system can improve the overall alignment and consistency of the radiographic results even in the hands of experienced surgeons. Additional benefits like reduced postoperative drainage are also observed.

I137/SC

Early Experience in Free Tissue Transfer in Head and Neck ReconstructionCM LIM¹, J LIM², KSL TAN¹*¹Department of Otolaryngology—Head and Neck Surgery, National University Hospital, Singapore, ²Department of Surgery, National University Hospital, Singapore*

Aim: The aim of this study is to retrospectively review our early experience with free tissue transfer in the reconstruction of head and neck defects following extirpation of head and neck cancers in a tertiary hospital in Singapore.

Methods: Medical records of patients were traced and a total of 25 patients had undergone free tissue transfer between June 1998 and October 2003. An overall descriptive analysis was carried out looking at the following outcome measures: length of hospitalisation, duration of ICU stay, readmission and complication rate.

Results: There were 18 free radial forearm flaps, 4 rectus abdominis flap 2 free fibular osteocutaneous flaps and 1 free lateral arm flap. The mean length of stay was 12.6 days (SD 7.3) and mean stay in ICU was 1.8 days (SD 1.3). Two patients (8.3%) were readmitted within a 30-day period for flap related complications. Six patients (24%) developed flap related complications. Two patients developed pharyngocutaneous fistula, 3 patients developed flap venous congestion and 1 patient developed minor donor site haematoma. Salvage anastomotic revision was performed in all the 3 congested flaps and 1 of the flaps was successfully revived while the other 2 flaps were lost. Hence, our flap success rate was 92%.

Conclusion: Our early experience shows that free tissue transfer can be a safe surgical option in the reconstruction of head and neck defects. We believe that our initial success will further improve with increase in experience and technical skills.

I138/SC

Role of Fine Needle Aspiration Cytology in Parotid Gland MassesCM LIM¹, J THAY², KSL TAN¹*¹Department of Otolaryngology—Head and Neck Surgery, National University Hospital, Singapore, ²Medical, Faculty of Medicine, National University of Singapore, Singapore*

Aim: The objective of the study is to evaluate the accuracy of fine needle aspiration cytology (FNAC) in the diagnosis of parotid masses.

Methods: A retrospective review was conducted to examine the clinical records of patients who underwent parotidectomies between January 1997 and April 2004 in the Department of Otolaryngology-Head and Neck Surgery at the National University Hospital, Singapore. A total of 99 patients were found and 91 patients had preoperative FNAC which comprised our study population. The preoperative cytologic and final histologic results were correspondingly analysed.

Results: Histological diagnosis revealed 10 (11.0%) malignant tumours, 74 (81.3%) benign tumours and 7 (7.7%) non-neoplastic lesions. Cytology was non-diagnostic in 10 cases (11%). The overall accuracy for FNAC was 93.8% (76/81). Sensitivity and specificity for diagnosing malignant and benign tumours were 80%, 100% and 98.5%, 87.5% respectively. 83.7% (62/74) of benign tumours were accurately typed on FNAC. However, only 30% (3/10) of all malignant tumours were accurately typed.

Conclusion: FNAC is useful in the preoperative assessment of parotid tumours. Although it may not accurately type the malignant tumours, the preoperative diagnosis of malignant tumours may allow for appropriate surgical planning for the surgeon.

I139/SC

Accuracy of Intraoperative Frozen Section Evaluation in Salivary Gland Surgery

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Aim: Intraoperative frozen section evaluation of salivary gland tumours is an important tool in determining the extent of surgery. Frozen section results have been reported to be very accurate for benign tumours, though less so for malignant ones. We review our experience.

Methods: Retrospective analysis of all salivary operations done over 3 years in the Department of Otorhinolaryngology, Tan Tock Seng Hospital.

Results: Between July 2001 and June 2004, 91 patients underwent salivary gland surgery in our department. Of these, 77 had frozen sections done, of which 53 had benign tumours, 11 had malignant epithelial tumours, 4 had lymphoid lesions and 9 had non-neoplastic conditions. Of the 11 epithelial malignancies, the accuracy of frozen section was 45.5%. There were 3 incorrect diagnoses of a benign tumour, and 3 deferred diagnoses. Two of the false negatives had a clinical impact, requiring a second procedure. Of the benign tumours, 51 of 53 were correctly identified giving an accuracy of 96.2%.

Conclusion: Frozen section evaluation is highly accurate for benign tumours of the salivary glands. However, for salivary malignancies, frozen section sensitivity and specificity is below 50%.

I140/SC

Extended Radical Neck Dissection in Nasopharyngeal Carcinoma

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Aim: Radical neck dissection (RND) is the treatment of choice for isolated neck recurrences following radiotherapy for nasopharyngeal carcinoma (NPC). With this approach, the historical regional control and cure rates are good. However advanced neck recurrences require major extension of the classic RND for extirpation. Whether this extended surgery, with its greater morbidity, achieves meaningful neck control and survival is uncertain. We review our experience with extended RNDs for NPC.

Methods: Between March 1995 and March 2004, 11 of 29 patients who underwent neck dissection for nodal recurrence in NPC required extended RNDs. These extensions included the resection of adjacent muscle, cranial nerves, parotid gland, ear canal and temporal bone, spinous processes, skin and the external carotid artery in various combinations. Four patients required myocutaneous flaps for coverage. Five patients received additional adjuvant radiotherapy postoperatively.

Results: After a mean follow-up of 17 months, only 1 of 10 analysable patients (10%) developed neck recurrence. Six patients are presently alive and disease-free, 3 have died of disease and 2 have died of other causes. The actuarial 3 year disease-free survival is 36.7%. This is comparable to

historical data for classic RNDs employed for more limited neck recurrences.

Conclusion: The local control and survival achieved by extended RND for advanced neck recurrences in NPC is encouraging. While extended surgery undoubtedly increases treatment morbidity, the ability to control advanced disease makes this approach worthwhile.

I141/SC

Diagnosis of Residual and Recurrent Neck Disease in Nasopharyngeal Carcinoma

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Aim: Following radical radiotherapy for nasopharyngeal carcinoma (NPC), diagnosing residual or recurrent neck disease can be difficult. Patients with complete resolution of nodal disease may later relapse in the neck. Conversely, patients with clinical or radiological evidence of persistent or recurrent neck disease may not have viable tumour on neck dissection. Computer tomography (CT) scans and fine needle aspiration cytology (FNAC) are frequently used to aid diagnosis of residual or recurrent neck disease. We review our experience with these investigations.

Methods: Between March 1995 and March 2004, 29 patients with previously treated NPC underwent neck dissection for nodal disease, 12 patients for residual disease and 17 patients for recurrent disease. We reviewed their pre-operative imaging and cytology results and compared them with the final histopathology.

Results: Five of 12 (41.7%) patients with residual nodes, and 10 of 17 (58.8%) patients with recurrent nodes had viable tumour on histology. FNAC results correlated poorly with final histology, with false positive and negative rates 25% and 100% for recurrent cases, and 50% and 40% for residual cases respectively. CT scan findings were only slightly more accurate. For recurrent cases, the false positive rate was 22%, while the false negative rate was 33%. For residual cases, the false positive rate was 55%.

Conclusion: The usefulness of CT scans and FNAC in diagnosing residual and recurrent neck disease in NPC is suspect. There is a need to evaluate the accuracy of newer investigations like positron emission tomography (PET) scans in these difficult situations.

I142/SC

Phase II Study of the American Brachytherapy Society Guidelines for the Use of High Dose Rate Brachytherapy in the Treatment of Cervical Carcinoma Implementation in an Asian Society

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Aim: In 2000, the American Brachytherapy Society (ABS) published incompletely evaluated guidelines for curative chemoradiation and high dose rate (HDR) brachytherapy for cervical cancer: our aim was to assess guideline tolerability in an Asian population.

Methods: From 2000, all stage I-IVa cervical carcinoma patients were treated following ABS guidelines. Early disease (FIGO stage I/II

Results: Nineteen of 21 (90%) patients (8 early, 13 advanced stage) received planned radiation, and 86% received planned chemotherapy. Median follow up was 18 months (range, 5 to 43 months). Two-year actuarial survival (S) was 81% and disease free survival (DFS) 76%. S/DFS for early and advanced stage was 88%/86% and 77%/60% respectively. Eighty-six per cent achieved a complete response (CR), 14% partial response. For those in CR, there were no local failures. Acute cystitis occurred in 24%, proctitis 5%, and gastroenteritis 48%. Late cystitis occurred in 10%, gastroenteritis 5%, and genitourinary fistula (in the presence of progressive disease) in 5%. No grade 3/4 treatment-related toxicity occurred.

Conclusion: The ABS guidelines were well-tolerated and efficacious in our study, although longer follow-up is required. Further studies are warranted to validate safety and efficacy of the recommendations.

I143/SC

Prospective Phase II Trial of Concomitant Boost Radiotherapy for Stage II Nasopharyngeal Carcinoma an Evaluation of Response And Toxicity

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Aim: Stage II nasopharyngeal carcinoma (NPC) treated with conventionally fractionated radiotherapy results in loco-regional control of around 80%. This report aims to document the outcome of Stage II NPC patients treated with external beam radiotherapy delivered using an accelerated concomitant boost (C-boost) schedule.

Methods: Twenty-five 1997 UICC TNM Stage II NPC patients were enrolled and analysed in this report. The primary tumor and clinically involved nodes received a total dose of 72 Gy in 42 fractions. C-boost for gross disease consisted of 18 Gy in 12 fractions commencing on day 19, and delivered at least 6 hours after the first dose. Patients were assessed for treatment response, survival and toxicity.

Results: With a median follow-up of 24 months, only 1 patient had pathologically confirmed local recurrence, necessitating IMRT. Two patients developed distant metastases for which they received chemotherapy. One patient died from systemic disease after refusing treatment for persistent neck lymphadenopathy. The 2-year loco-regional control rate, overall survival and disease-free survival rates were 96%, 96%, and 88% respectively. All patients experienced some degree of treatment-related acute and/or late toxicity. However, the toxicity profile was comparable to that seen following standard fractionation. Acute or late toxicities directly attributable to C-boost were not observed.

Conclusion: This concomitant boost radiotherapy regimen administers a substantially higher biologically effective dose compared with conventional radiation schedules. Preliminary loco-regional control and survival rates are promising with no significant acute and/or late toxicities.

I144/SC

Correlation Between Decreased Intracranial Pressure After Mannitol Administration and Improved Brain Oxygenation

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Aim: To analyse changes in PtiO₂ and ICP in patients with acute brain injury. Obtain correlation between stable decrease in ICP (≥ 30 minutes) following mannitol administration and corresponding changes in brain oxygenation.

Methods: We studied 57 mannitol administrations in 17 patients with severe head injury. These patients had intraparenchymal ICP monitor and Neurotrend inserted via a multi-lumen bolt. ICP and O₂ point values were extracted on a 5-seconds time interval via the multiparameter monitoring probes. For each administration, we studied 5 consecutive 30-minute timeframes and determined their average ICP and O₂ values. The timeframe with the minimum average ICP post-administration along its corresponding O₂ was identified and both values subtracted from baseline references for that sample to obtain ICP and O₂ changes.

Results: We presented the analysis of 35 mannitol administrations, mean age 44 years, median GCS on admission = 10, mean baseline ICP = 21.479 mmHg, mean baseline O₂ = 33.862 mmHg, Mean ICP change = -3.46 mmHg (± 4.089); Mean O₂ change = +2.078 mmHg (± 10.079). We compared ICP and O₂ changes using Pearson's correlation coefficient, to obtain $r = 0.615$, significant level at 0.01 (2-tailed). 18 samples with baseline ICP 20 mmHg, $r = -0.583$ significant level at 0.05 (2-tailed).

Conclusion: The study shows moderate to good relationship ($-0.5 < r < -0.75$) between decreased ICP and increased brain O₂ values following mannitol administration irrespective of baseline ICP, suggesting mannitol has the physiological effect of decreasing ICP and the potential of improving brain oxygenation.

I145/SC

The Patients' Perspective and Attitudes Towards Nipple Reconstruction

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Aim: Nipple reconstruction (NR) remains the most common part of post-mastectomy reconstruction. To evaluate 1) Patients' motivations to undergo NR. 2) Patients' attitudes toward NR.

Methods: Fifty-two patients treated in the plastic surgery unit (National University Hospital, Singapore) following breast reconstruction were contacted through telephone. Thirty-nine responded, 1 rejected and 12 were non-contactable. The subjects stated their main motivations/disincentives towards undergoing NR. Questions that make up the attitude score (AC) include aspects such as self-esteem, physical relationship, economic concerns, necessity of procedure and pain.

Results: 1. Group A (14 responded subjects who had undergone, or are going to do NR) – 5 regarded “wanted to have a complete breast” as the main motivation; followed by “doctor's influence” (4), “no particular reason” (3) and “no harm trying” (2). 2. Group B (25 responded subjects who had not undergone, or did not intend to undergo NR) – 10 regarded “not a necessary operation/does not see the need” as the main disincentive; followed by “does not want another operation” (7), “current body condition does not allow for surgery” (4), and others (3). 3. Group A's average AC was 15.8/20, while group B's was 12.2/20.

Conclusion: 1. The main reason for NR was “wanted to have a complete breast”. 2. Group A subjects generally believe NR improves self-esteem and physical relationship with partner. 3. Questioning the need and necessity for NR was the main disincentive in group B subjects. 4. Group B subjects generally believe NR wastes money, is not worth the time or effort, causes further pain and is unnecessary.

I146/SC

Body Image Concerns of Asian Women After Mastectomy and Breast Reconstruction

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Aim: Breast cancer is one of the most common cancers in women. The psychosocial impact of breast reconstruction is extensively studied in the Western population. There is a relative paucity of comparable data in Asian women. Body image has been a key determinant of psychosocial health. **AIM:** To assess body image concerns of Asian women after mastectomy and breast reconstruction.

Methods: Fifty-three patients from the period of July 1999 to December 2003 who had undergone mastectomy and breast reconstruction in National University Hospital were followed up. Exclusion criteria are: 1. Not of Asian heritage (1); 2. Deceased (1). Of the 53, 2 were excluded, 1 rejected and 12 were non-contactable. Of the remaining 38, 30 (78.9%) Chinese, 5 (13.2%) Malay and 3 (7.9%) other Asian heritage (Filipino, Eurasian, Indian). Phone interviews were conducted using a 10-item body image scale (BIS). Each item was evaluated individually and the 10-item scores were summed to produce overall summary scores for each patient (range 0-30). Zero indicates no symptoms/distress and higher scores represent increasing distress.

Results: The average summary score is 6.32. The individual item scores are higher in cognitive items than in behavioural items. Affective items scores are split with “feeling less physically attractive” being highest among the 10 items and “feeling less sexually attractive” the 9th.

Conclusion: 1. On the BIS, Asian women scored lower (average 6.32). 2. Although they are displeased with the physical effects of the treatment, they are not as affected emotionally or behaviourally.

I147/SC

The Role of Empirical Treatment with Antibiotics in Patients with Elevated PSA – A Prospective Cohort Study

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Aim: Patients with elevated serum PSA undergo transrectal ultrasound-guided (TRUS) biopsy of the prostate to exclude prostate cancer. PSA is also elevated in prostatitis and BPH. We evaluate whether an empirical course of

antibiotics would identify patients with prostatitis, for whom TRUS biopsy would be unnecessary.

Methods: This is an ongoing prospective study of men aged 50-75 years, with PSA between 4-100 ng/ml. Patients are given a 4-week course of antibiotics followed by repeat PSA measurement before TRUS biopsy. PSA decline is significant if it exceeds biological variation of 15%.

Results: Ninety patients have completed the study. Mean age was 64 years, and median baseline PSA was 7.9 ng/mL. Thirteen prostate cancers were detected. The median baseline PSA of patients with and without prostate cancer was 7.8 and 7.9 ng/ml respectively. The median PSA change of patients with and without prostate cancer was +6% and -9% respectively; the difference in PSA change was not significant ($P = 0.082$). Thirty (33%) patients had significant PSA decline after antibiotic treatment. This is attributed to treatment of underlying prostatitis; 2 of these patients had concomitant prostate cancer. Sixty (67%) patients had no significant PSA decline; out of which 11 had prostate cancer. The difference in cancer detection rate between both groups was not significant ($P = 0.206$).

Conclusion: There is no role for empirical antibiotic treatment to differentiate patients with and without prostate cancer. Patients with elevated PSA should be biopsied if clinically indicated.

I148/SC

Testicular Infarction Following Suppurative Epididymo-orchitis: A Persistent Clinical Dilemma

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Aim: Suppurative change with testicular infarction is an extremely rare complication following failed antibiotic therapy for epididymo-orchitis (EO). We report a series of 6 cases diagnosed over a 10-year period.

Methods: Operative records of all non-oncologic orchidectomies performed over a 10-year period from June 1994 to May 2004 were reviewed and correlated with final histological diagnoses. Six cases of testicular infarction following suppurative EO were identified. Clinical records and histological sections were reviewed for presentation, risk factors, histopathological profile, management and prognosis. A PubMed search was performed using key words “infarction”, “necrosis”, “epididymo-orchitis” and relevant articles reviewed.

Results: The mean patient age was 43 (range, 19-76) years. Presenting symptoms include severe scrotal pain and swelling unresponsive to antibiotic therapy. Absence of testicular blood flow and abscess formation was documented on Doppler ultrasonography, and the mean duration from onset of symptoms to diagnosis of threatened testicular viability was 14 (range, 4-90) days. Possible predisposing factors included diabetes (2), end-stage HIV (1) and hypospadias (1). Orchidectomy was performed for 5 of 6 cases at exploration. Gonadal preservation was attempted in 1 case with cord and scrotal decompression, but the patient exhibited delayed infarction requiring subsequent orchidectomy. Microbiology results of responsible pathogens were not available. Postulated mechanisms include lymphatic and venous congestion from severe epididymitis causing vascular compromise and venous thrombosis.

Conclusion: Suppurative change with testicular infarction following EO is a rare but distressing outcome. Early surgical decompression in at-risk patients may improve gonadal salvage.

I149/SC

Mesh Versus Non-repair for Ventral Hernias in the Emergency Setting

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Aim: To compare ventral hernia repair in an emergency setting, with mesh repair versus a non-mesh repair.

Methods: In a comparative retrospective study of 29 patients with ventral hernias in a single tertiary institution over a 6-year period, we analysed their demographics, pre-morbid characteristics, types of hernia and their surgical procedure, the post operation complications and recurrences.

Results: All patients presented with incarcerated ventral hernias. Thirteen patients had emergency hernia mesh repair (group A). Seventeen patients who had non-mesh repair (group B). Twenty-six (90%) of all patients were female. There was no difference in age between the 2 groups (mean age was 63 years). Four patients (23%) of patients in group B had small bowel resection versus 3 patients (25%) of patients in group A. One patient in group A developed a wound haematoma and another developed an infected mesh which required removal of the implant. There was no mortality in this group. In group B, there were no wound-related problems but 1 patient died after she presented late with small bowel infarction and had bowel resection. One patient (8%) in the group A developed recurrence of a hernia as compared to 4 patients (23%) in group B.

Conclusion: Emergency mesh repair for ventral hernias decreases recurrence rates. Selection of their use is important and one may suggest their usage in a non-contaminated environment and when bowel resection is not involved.

I150/SC

Adjuvant Chemoirradiation After D2 Gastrectomy for Gastric Cancer

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Aim: Postoperative adjuvant chemoirradiation has been shown to be effective for gastric cancer in a recent multicentre trial. The role of adjuvant therapy for patients with D2 gastrectomy remains controversial. To determine tolerability of adjuvant chemoirradiation after D2 gastrectomy.

Methods: The data of patients who received chemoradiotherapy following D2 gastrectomy were prospectively collected. The adjuvant treatment consisted Leucovorin 5FU and radiotherapy. The side effects of the adjuvant treatment were measured using the Common Toxicity Criteria.

Results: Between 1 June 2000 and 31 August 2002, 37 patients were subjected to this treatment protocol after D2 gastrectomy. Twenty-nine patients completed the chemoirradiation regime. All patients who could not complete the regime cited the reason of intolerability and half of them occurred in the 5th course. A total of 10 patients were admitted to hospital during the course of chemoirradiation. Gastrointestinal disturbances of diarrhoea and vomiting were the most common side effects (78.4%), of which one patient was admitted. Six patients were admitted for neutropenic fever. Another was admitted after he developed a deep vein thrombosis. No patient developed suffered major toxic effects (grade 3 and above).

Conclusion: Seventy-eight per cent of patients completed the chemoirradiation regime reflecting good tolerability. None of the patients suffered major toxic effects (grade 3 and above). Further studies have to be done to investigate the effect of D2 gastrectomy plus adjuvant chemoradiotherapy on the survival of these patients with resectable adenocarcinoma.

I151/SC

Infrapopliteal Angioplasty in Diabetics for Limb Salvage

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Aim: In the revascularisation of critical ischaemia, femoral-distal bypass has given consistent results in terms of durability and limb salvage. Angioplasty of the tibial vessels has been used as an alternative means of revascularisation and we review our results in terms of efficacy in limb salvage, wound healing and its patency rates.

Methods: All patients admitted to our hospital with the diagnosis of non-healing ulcer with critical ischaemia are entered into a pathway. Ankle and toe pressure indices with duplex ultrasonography were used to reveal the extent of the atherosclerotic disease. Patients were offered angioplasty as a first option with an intent to treat basis. Pre and post procedural ankle indices were monitored. Successful angioplasty was defined as re-establishment of straight line flow from the femoral vessels to at least a single run-off vessel in the foot.

Results: Seventy-two subjects were analysed for this study. Most (76%) of

these patients were diabetic. Seven major amputations were performed giving an immediate limb salvage rate of 90%. There was a mean improvement of ankle pressure indices of 0.23 (SD $\pm$ 0.22) in the group where angioplasty was successful. There was a 30-day mortality of 1.4% (1 patient).

Conclusion: Angioplasty offers a viable option for revascularisation in the critically ischaemic limb patient. Short-term patency and limb salvage rates are compatible with distal bypass. There is a tendency for recurrence but frequent re-intervention results in acceptable secondary patency and limb salvage rates.

I152/SC

Gastric Stapling Banding for Morbid Obesity

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Aim: The results of bariatric surgery have been well documented in large series in the West. In Asia, where obesity is less rampant, such surgery has been correspondingly less frequent, and there is a dearth of information on bariatric surgery on Asians.

Methods: This paper documents the outcome of a personal series of 40 patients who underwent gastric stapling and banding from 1987 to 2003 in Singapore.

Results: From 1987 to 1997, 26 patients underwent open gastric stapling [gastric bypass (4), vertical banded gastroplasty (22)]. Initial mean BMI was 43.3 kg/m². At half, 1, 2, 4 and 8 years after surgery, mean BMI was 35.2, 31.9, 31.2, 31.1 and 34.1 kg/m². Mean initial weight was 127.2 kg. % EWL was 42.2, 56.2, 56.9, 56.3 and 48.3%. From 1999 to 2003, 14 patients underwent lap banding, 11 by laparoscopy. Initial mean BMI was 42.9 kg/m². At ½, 1 and 2 years, mean BMI was 38.9, 36.6, and 32.6 kg/m². Mean initial weight was 122.6 kg. Percent EWL was 26.6%, 38.8% and 59.2%. There was no operative mortality. One patient, following perigastric insertion of lap band developed band slippage. We have since adopted the newer technique of combined pars flaccida and perigastric dissection and have had no band slippage in the last 6 patients.

Conclusion: Our results of safety and low operative morbidity as well as pattern and magnitude of weight loss following gastric stapling and banding for morbidly obese patients in Singapore appears to be similar to Western experience.

I153/SC

Laparoscopic Adjustable Gastric Banding: The Singapore Experience

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Aim: The incidence of overweight, obesity and morbid obesity is on the rise in Asian countries. The demand for Bariatric surgery is on the rise as it has been shown to be the only reliable means in effecting weight loss in morbid obesity. We have performed 100 laparoscopic adjustable gastric bandings (LAGBs) to date and are the only group in Singapore with such a wide experience. LAGB is superior to other surgical options in being a purely restrictive surgical procedure.

Methods: The LAGB involves placing an adjustable silicone band in the upper part of the stomach to create a small pouch and the outlet of the pouch can be adjusted via a port buried in the fat. We use the pars flaccida technique.

Results: We have performed 100 LAGBs to date in an Asian populace with BMI >40 and BMI >32.5 with comorbidities. (ASBS, IFSSO, INNAME, MOH). Our age range was 26 to 50 (average 36.8) years with a M:F of 5:13 and a conversion rate of 2%. The weight loss ranged between 0.19 and 2.6 kg/week (average 0.78) and the BMI loss was about 0.07-1.12 per week. Selected comorbidities also showed remarkable resolution/improvement such as DM - 20%/80%, Hypertension - 35%/65% and knee pain - 60%/40%.

Conclusion: The Health For Life programme in Alexandra Hospital, Singapore, has estimated the prevalence of BMI >35 requiring bariatric surgical intervention to be approximately 1.6%. The LAGB is a very good surgical option in morbid obesity in the Asian population with minor changes in selection criteria.

I154/SC

Laparoscopic-assisted Colon and Rectal Surgery—Lessons Learnt from Early Experience

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Aim: Current evidence shows that laparoscopic bowel surgery is associated with significant benefits. We report our early experience in laparoscopic-assisted colorectal procedures in our Colorectal unit.

Methods: All (LAC) procedures performed between January 2000 and December 2003 were reviewed. An equal number of matched open procedures were accrued and analysed for comparison. All patients were managed on a standard carepath. Data was analysed using a statistical software package.

Results: Forty-two LAC procedures were performed. Seven were converted to open for various reasons. LAC procedures performed included 1 right, 5 left hemicolectomies, 9 anterior resections, 1 abdominal-perineal resection, 3 sigmoid colectomies, 11 colostomies and 1 Hartmann's procedure. Mean perioperative time (146 min vs 125 min, $P = 0.173$) was comparatively longer. Mean duration for analgesia (2.25 days vs 2.64 days, $P = 0.05$), mean length of stay (5.31 days vs 9.07 days, $P < 0.05$), mean time to commencement of diet (2.91 days vs 4.05 days, $P < 0.001$) and mean time to first bowel movement (2.57 days vs 4.10 days, $P < 0.001$) were all comparatively shorter. General morbidity rates (17.1% versus 21.4%, $P = 0.35$) were lower. No local wound complications were found in the LAC group. Patients who had procedures converted to the open method fared poorer.

Conclusion: LAC procedures performed in well-selected patients are associated with shorter hospital stay, quicker return of bowel function and less morbidity. Early experience should be acquired from performing technically simple procedures in patients with benign conditions before progressing to oncologic resections.

I155/SC

Advanced Breast Cancer in Malays

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Aim: To study the characteristics of locally advanced and metastatic breast cancer among local Malays.

Methods: The medical records of patients with breast cancer seen in the department from 2000 to 2003 were reviewed retrospectively.

Results: 622 women were diagnosed with invasive breast cancer in our department from January 2000 to November 2003. 134 patients had locally advanced breast cancer (LABC) or metastatic breast cancer (MBC), and 488 patients had early breast cancer. 524 patients were Chinese, 60 Malays, 25 Indians and 13 others. Malays were found to be 2 times more likely to have either LABC or MBC compared to the other races ($P = 0.024$, OR 1.93). Patient and tumour characteristics between the Malays and Chinese were compared. There was no significant difference in age, menopausal status, parity or family history between the Malays and Chinese. Neither was there any significant difference between tumour histology, size or grade, although the tumours in Malays were 3.6 times more likely to be oestrogen receptor (ER) negative ($P = 0.026$). Tumours found in Malays are therefore not likely to be more aggressive than those found in Chinese.

Conclusion: A lack of awareness and a delay in seeking treatment may account for the more advanced stage at presentation amongst Malays. There is therefore a need to promote a greater awareness among the Malays, to encourage them to perform regular breast self-examination and to seek prompt medical attention when breast symptoms arise.

I156/SC

A Randomised Controlled Trial of Standard Dose BCG Versus Low Dose BCG and Interferon Alpha in Patients with Superficial Bladder Cancer

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Aim: To determine whether dose reduction of BCG combined with Interferon alpha (IFN α) could reduce toxicity and maintain the efficacy of BCG immunotherapy for bladder cancer.

Methods: 140 patients were recruited for a randomised, controlled double-blind trial comparing standard dose BCG (81 mg) with low dose BCG (27 mg) and low dose BCG (27 mg) with 10 MU IFN α . Patients received 6 weekly intravesical instillations of intravesical therapy, followed by a 6-week rest period before receiving a further 3 instillations. Toxicity symptoms were recorded using linear analog scale. Subjects underwent 3-monthly cystoscopy and urine cytology. Data were analysed using multiple logistic regression, nonparametric tests and Kaplan-Meier recurrence-free plots.

Results: In patients who received low dose BCG, with or without IFN α , there was significantly less fever $>38.5^{\circ}\text{C}$, dysuria, burning sensation, frequency, haematuria and lassitude. Overall, there was a statistically significant 30% reduction in both local and systemic toxicity in the low dose BCG arms. Only 126 patients were evaluable for efficacy analysis. For standard, low dose and combination therapies, the recurrence rates at 12 months and 24 months were 24%, 18% and 13% and 42%, 24% and 15% respectively. The mean time to recurrence was 51, 59 and 66 months respectively. The hazard relative ratio for recurrence in the combination and low dose arms were 0.49 and 0.70 relative to standard dose BCG.

Conclusion: Five-year data suggest that intravesical low dose BCG in combination with IFN α has lower toxicity and may have equivalent, if not superior efficacy compared to standard dose BCG in patients with superficial bladder cancer.

I157/SC

Emphysematous Gastritis – A Case Report and a Review of Literature

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Aim: Emphysematous gastritis is a severe, rare and unusual form of gastritis caused by infection of stomach wall with gas producing organisms. Predisposing factors are diabetes, alcoholism, corrosive ingestion and in immuno-compromised individuals. We report a case of emphysematous gastritis in a post hepatectomy patient, who responded to conservative management.

Methods: A 45-year-old Chinese lady, known hepatitis B carrier, with Child's A cirrhosis and a hepatocellular carcinoma in the right lobe was referred to our unit. She subsequently underwent a right hepatectomy. On POD 11, she became increasingly septic, jaundiced and encephalopathic. A CT of her abdomen showed the stomach wall to be oedematous, with air pockets within the thickened stomach wall. Cultures from the abdominal drain showed multi-resistant *Pseudomonas* and *Acinetobacter* sensitive to meropenem. She was treated conservatively with intravenous meropenem. The patient responded to conservative management and was discharged after 7 weeks.

Results: Emphysematous gastritis was first described by Frankel in 1889, about 15% of cases are in recent post abdominal surgery period. Diagnosis is usually based on the circumstances, clinical presentation and imaging investigations of CT/MRI. Reported mortality is about 60%, though some authors have reported 100% mortality in patients without surgical intervention. Associated perforation is an indication for surgery. Our patient settled on conservative management.

Conclusion: Though there are many advocates for immediate surgery, of recent, there has been a trend towards conservative management.

I158/SC

Evolving Role of Open Nephrectomy in the Age of Laparoscopic Nephrectomy

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Aim: We analysed the indications for open radical nephrectomy in our institution with a comprehensive laparoscopic programme.

Methods: We reviewed 29 laparoscopic and 15 open radical nephrectomies for renal cell carcinoma from August 2001 to June 2004 with regards to the choice of approach, operating time, length of stay and operative complications.

Results: Comparing open versus laparoscopic groups, median patient age was 62 vs 63 years, average tumour size on preoperative imaging was 9.8 cm vs 5.5 cm, average operating time was 177 min vs 243 min, average blood loss was 860 mL vs 647 mL, and median postoperative length of stay was 6 days vs 3 days respectively. We had 7.1% (2 cases) conversion to open surgery but had no other major morbidities and mortality. Open nephrectomy was performed in 34.1% and the indications were for large tumour (>13 cm) in 4, renal vein tumour thrombus in 4, surgeon preference in 3 (during our early laparoscopic experience), 1 attempted partial nephrectomy, 1 with local invasion of surrounding organs, 1 previous colonic resection with stoma and 1 with large lymph nodes encasing renal hilum.

Conclusion: Despite laparoscopic surgery having become routine at our institution in the past 3 years, open radical nephrectomy retains an important role in up to one-third of patients. The indications for open surgery are evolving as the rate of open surgery may be further reduced with increasing laparoscopic experience, use of intraoperative ultrasonography and acceptance of this relatively new procedure.

I159/SC

Orthotopic Liver Transplant in Patients with Liver Disease Complicated by Portal Vein Thrombosis

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Aim: Portal vein thrombosis (PVT) once considered a contraindication to liver transplant, however with recent advances, does not preclude successful orthotopic liver transplantation. It still has a substantial impact on surgical complexity, perioperative morbidity and mortality. The incidence of PVT ranges from 2% to 13% in reported literature.

Methods: At the National University Hospital, Singapore, we have performed 100 Orthotopic liver transplants since the inception of the programme. We had 1 patient with PVT complicating the liver transplantation procedure. The strategies used at the time of surgery include, A) Thrombectomy of portal vein till the junction of splenic vein (SV) and superior mesenteric vein (SMV) if vein/thrombus are soft. B) Thromboendarterectomy may be needed using endarterectomy spatulas and cannon strippers. C) Eversion endarterectomy is usually needed if the thrombus is organised. D) Distal portal vein only involvement can be managed by excision if recipient portal vein is of adequate length. Precise suturing with intimal approximation and occasional tacking of intimal flaps is mandatory PVT extending beyond the SMV and SV junction do need iliac vein grafts to bridge the portal vein or vena cava, portal vein transposition.

Results: At the National University Hospital, Singapore, we have performed 100 Orthotopic liver transplants since the inception of the programme. One of our patients had portal vein thrombosis adding to the complexity of liver transplantation procedure. Post transplant he did very well and was discharged home on day 17. The delay was due to control of his diabetes and learning curve in his immunosuppression medication.

I160/SC

Necrotising Pancreatitis Management in Concordance with IAP Guideline

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Aim: Introduction: We report a case of acute necrotising pancreatitis with a Ranson's score of 7 and a CT severity index of 9. This patient has a morbidity and mortality rate of as high as 100%.

Methods: 68-year-old Chinese lady presented with gallstone pancreatitis of 1 day duration. She was started on intravenous meropenem. By day 5 she developed SIRS. CT demonstrated near total necrosis of pancreas. She became septic by day 12. Computed tomography on day 16 showed a large infected pseudocyst. Pancreatic debridement and necrosectomy, cholecystectomy and insertion of feeding jejunostomy, were performed on day 20.

Results: The patient had 1 day of ICU stay post operatively. Post operatively

patient had a gradual and unremarkable recovery. Our team managed this case in concordance with most of the recommendations. We feel that following IAP guidelines helped in the successful outcome for our patient, who had high predicted rate of morbidity and mortality.

Conclusion: IAP guidelines are useful and helpful in the management of most patients with severe acute pancreatitis, though individual variations in patient circumstances have to be borne in mind, and treatment modified as appropriate. Imaging, clinical status and clear demarcation of the necrotic pancreatic tissues are better predictors for the need for surgery as also the clinical status of the patient.

I161/SC

Elasticity Imaging of Breast Tumours

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Aim: Some pathological conditions, such as malignant tumours, manifest themselves as changes in the tissue's mechanical stiffness. This is the basis for palpation. Due to increased stiffness, when vibration is applied to tumour-bearing tissue, the movement of the tumour is less than that in the surrounding tissue. Modern ultrasound systems can be used to reveal how tissues move in response to vibrating force. Several techniques for imaging tissue elasticity have been proposed: compression elastography, transient elastography and sonoelastography. Visualisation of elastic properties using ultrasound imaging to display variations in tissue motions is known as sonoelastography.

Methods: A vibration assembly consisting of 3 cm disc driven by an audio-speaker, powered through frequency generator and musical amplifier was used as tissue contact element to induce low frequency sinusoidal oscillations. An ultrasound probe coupled with acoustic gel scanned tissue vibrations under power Doppler mode. Experiments were conducted on breast phantoms and with ethical clearance the breast in patients were also examined using conventional B-mode and sonoelastography, just prior to breast surgery.

Results: In breast phantom, an iso-echoic lesion on B-mode scan showing no detectable contrast with the neighbouring tissue appeared as light coloured region to surrounding bright coloured area on power Doppler scan. *In vivo* different breast lumps revealed varied colour patterns. Some as colour void areas, other as colour filled regions associated with B-mode speckles.

Conclusion: The preliminary findings suggest that our technique could be used as an adjunct imaging method to conventional B-mode scanning for detection and diagnosis of lumps.

I162/SC

Different Endoscopic Approaches in Palmar Hyperhidrosis: A Critical Appraisal

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Aim: The aim of our study was to evaluate the results of thoracoscopic sympathectomy for severe upper limb hyperhidrosis using different surgical techniques: needlescopic, single port techniques and standard 5 mm thoracoscopic surgery (VATS).

Methods: Between May 1996 and December 2003, 90 consecutive patients underwent thoracoscopic sympathectomy procedures to treat bilateral hyperhidrosis (180 procedures). 50 patients underwent needlescopic procedures and 28 patients underwent single port procedure and 12 patients underwent VATS. Operative results, complications, and recurrence were determined by reviews of hospital and office charts and by follow-up assessments in the outpatient clinic.

Results: Ninety patients (52 female, 38 male; mean age, 26.12 years) presented with primary palmar hyperhidrosis. Twenty-eight patients had axillary hyperhidrosis while 26 patients had associated plantar hyperhidrosis as well. The average length of hospitalisation was 1.38 days (1-4 days). At 23.67 months (CI 95%) follow-up: 17 (19%) patients reported bothersome compensatory hyperhidrosis and 2 patients considered it severe. All patients had relief of palmar hyperhidrosis, 94.8% reported good results, and 2.6 % reported satisfactory and poor results respectively. All postoperative complications were transient, 2 patients had chest tube inserted for significant pneumothorax and 8 patients had transient subcutaneous emphysema, 2 patients reported persistent chest pains. There was no significant difference

in the hospital stay, analgesia requirements and complications between needlescopic and single port techniques.

Conclusion: Thoracoscopic sympathectomy using a needlescopic and single port techniques effectively treats hyperhidrosis and is associated with short hospital stays, high patient satisfaction rates, and low rates of compensatory hyperhidrosis or other complications.

I163/SC

Endoscopic Totally Extraperitoneal Inguinal Hernia Repair – A Single Centre Experience

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Aim: Laparoscopic hernia surgery has maintained its role because of the benefits to patients that are evident when compared to open repairs, as reported in many published randomised controlled trials. We review our experience of endoscopic totally extraperitoneal hernia repair (TEP) in a major teaching hospital.

Methods: A review was undertaken of 280 consecutive patients who underwent TEP for inguinal hernia between 1998 and 2004 at the National University Hospital, Singapore.

Results: The 280 patients had 350 hernia repairs (234 unilateral and 116 bilateral hernias). The mean age was 51 years and 89% were men. The overall mean operative duration was 70 minutes; bilateral repairs took 27% longer than unilateral repairs. Five patients had conversion to open surgery, and 16 patients developed minor complications (groin seroma). 9 patients (3%) developed hernia recurrence, but there was no recurrence detected in the last pts (52%). The recurrence rate was higher when the mesh was not anchored (5 of 45 patients; 11.1%) than when the mesh was anchored (3 of 235 cases; 1.3%). The mean inpatient hospital stay was 1.4 days, and of the last 96 cases, 70% were performed as outpatient.

Conclusion: Endoscopic TEP is a viable alternative to open hernia repair. To achieve good results, adequate cases should be performed to overcome the learning curve, and that the mesh should be anchored to the inguinal floor.

I164/SC

Laparoscopic Versus Open Ventral Hernia Mesh Repair: A Prospective Study

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Aim: We compared laparoscopic ventral hernia repair against open ventral hernia repair to determine if laparoscopic repair is safe and effective in our setup.

Methods: Data of 71 consecutive patients with ventral hernia mesh repaired by either laparoscopic or open technique from August 2001 to November 2003 were collected.

Results: Open repair was done in 37 patients and 34 had laparoscopic repair. Mean hernia size for the laparoscopic group was 93 cm² versus that for open of 55 cm², mesh sizes 216 cm² versus 110 cm² and mean operating time was 96 minutes versus 116 minutes respectively. A statistically significant difference ($P = 0.002$) was seen between postoperative stay, laparoscopic 2.29 ± 0.97 days versus 3.81 ± 2.55 days in open. Postoperative pain score (VAS) was significantly different at 72 hours ($P = 0.017$). There was no conversion to open repair. In the laparoscopic group 1 patient had seroma and 1 prolonged ileus versus 4 seromas and 5 patients with prolonged ileus in open group. Two patients in the open group 2 required removal of mesh for infection. Mean follow-up time is 11.2 ± 9.81 months for laparoscopic patients versus 13.76 ± 7.54 months for open group. Recurrence rate of 2.94% is noted in the laparoscopic group, as compared to a much higher (10.81%) in the open group.

Conclusion: In our experience, laparoscopic ventral hernia repair is associated with decreased operating time, less postoperative stay, higher patient satisfaction rate, and lesser complications.

I165/SC

A Six-month Review of Ambulatory Surgery in Patients with Breast Cancer in Tan Tock Seng Hospital

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Aim: To evaluate the feasibility and safety of performing simple mastectomy with axillary clearance (SMAC) and wide excision with axillary clearance (WEAC) as ambulatory surgery.

Methods: Medical records of 109 patients who underwent SMAC and WEAC from December 2003 to May 2004 were reviewed retrospectively.

Results: The routine practice in our department has been to admit patients following SMAC or WEAC. In March 2004, our department started a 23-hour ambulatory surgery (AS23) programme. Twenty-nine patients underwent AS23 or day surgery (DS) from December 2003 to May 2004. These patients were discharged with pressure dressing over the wound and a single drain in the axilla. The breast nurse will remove the pressure dressing 2 days postoperatively and remove the drain 6 days postoperatively. The mean age of these patients was 49.5 ± 8.4 years. This was significantly younger than the mean age of patients who stayed as inpatients (57.0 ± 13.1 years). These patients also had less severe co-morbidities. 24.1% of those who underwent ambulatory surgery had a SMAC procedure done as compared to 68.8% of inpatients. A significant number of patients who were admitted for more than 24 hours were from nursing homes or had poor social support. There was no significant increase in local wound complications between the 2 groups.

Conclusion: Younger patients with minimal co-morbidities and good social support are suitable candidates for ambulatory surgery, which can be performed without significant postoperative morbidity.

I166/SC

Laparoscopic Left Hepatectomy Case Report and Review of Literature

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Aim: Laparoscopic surgery has seen major advancement in techniques and refinement in the instruments used, making it now more feasible to explore the place of laparoscopic resection of the liver. We here report a case of a patient with multiple liver adenomatosis in segments 3 and 4, who underwent a successful laparoscopic left hepatectomy for dominant nodule in segment 4.

Methods: A 50-year-old Chinese male presented with left lower limb swelling. Biopsy of lesions suggested high grade sarcoma. Staging CT revealed several lesions in segment 3 and 4 of liver. CT guided biopsy of liver tissue revealed adenoma. He underwent laparoscopic left hepatectomy followed by left below knee amputation.

Results: The patient had a rapid postoperative recovery. He was ambulatory with assistance on postoperative day 1. On postoperative day 4, he was discharged home.

Conclusion: Laparoscopic hepatectomy is a safe and feasible operation with careful patient selection. Besides a reduced postoperative analgesic requirement, shorter time interval to oral intake and reduced hospital stay, there is a faster improvement in the serum transaminase levels post operatively. Localisation of the lesions is of crucial importance and peripheral lesions in segment II-IV are more feasible for laparoscopic approach for resection. With experience, more cases and more complex lesions can be offered laparoscopic hepatectomy.

I167/SC

Evaluation of Renal Vasculature for Living Renal Donors – Comparing Computed Tomographic Angiography with Conventional Angiography

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Aim: To compare the reliability and accuracy of multi-sliced contrast enhanced computed tomographic angiogram (CT) to that of conventional angiogram (Angio) in detecting vascular abnormalities in living renal donors who had undergone transplant nephrectomy.

Methods: From January 2000 to April 2004, operation was performed in 33 renal donors. Prior to nephrectomy 10 had CT and 23 had Angio. Comparisons

were made between intraoperative findings to the CT or Angio findings. Cost benefit analysis was done.

Results: Of the 10 patients who had CT, 6 were reported as normal and 4 had minor abnormalities that should not preclude graft nephrectomy. Intra-operatively 1 of the 6 with normal CT had minor vascular abnormalities detected that did not preclude graft nephrectomy. Of the 4 with abnormal CT, no additional findings were noted and graft nephrectomy was performed. Of the 23 patients who had angiograms, all were normal. Intra-operatively no abnormalities were detected in 17 patients (74%) and 6 were abnormal (26%). Of the 6 who were abnormal, none were considered to be major. One of the donors did not undergo donor nephrectomy due to structural abnormalities of both kidneys that was not detected on the intravenous urogram. One patient underwent an asthmatic attack following the Angio.

Conclusion: CT is at least equal to, if not better than Angio for pre-operative vascular evaluation of living renal donors. It has advantages of less invasiveness, cost savings, lower morbidity and increased level of renal assessment.

I168/SC

Management of Stricture Urethra

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Aim: We determined whether optical urethral reconstruction is effective for the treatment of urethral stricture. All patients with stricture urethra were subjected to optical urethrotomy and its mid-term outcome was analysed

Methods: During a 3-year period 192 men underwent total of 322 urethrotomies. Seventy-nine lesions were in the bulbar urethra, 21 in the posterior and whole urethra was involved in 17 cases. Sixty-four were in anterior urethra and in the rest of the 11 cases site was not mentioned. Guided optical urethral reconstruction consisted of optical urethrotomy performed with a metallic bougie introduced proximally through the suprapubic catheter site and into the proximal urethra with the index finger of the operator in the rectum in a few cases.

Results: Of the patients 112 (59%) were cured after 1 procedure, whereas another 33 (17.1%) required 2 procedures. The remaining 32 (16.5%) patients required 1 to 6 urethrotomies (mean 4) except 1, who required 9, 6 patients underwent urethroplasty, 3 endoscopic rail road and 6 patients lost to follow up. Hematuria occurred in 2 of the patients, extravasation of urine in 2, and 2 patients developed urethrorectal fistula, 1 heals spontaneously.

Conclusion: Our procedure is effective, simple, safe, does not require special or sophisticated guiding instruments, which are necessary for previously described techniques. It can be performed with or without use of a bougie depending on the extent of the lesion and skill of the surgeon. The outcome can be judged from the symptomatic response of the patient, and flow studies and urethrography are not mandatory during routine follow-up.

I169/SC

Post Polymerisation Shrinkage Associated with Different Light-Curing Regimens

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Aim: Polymerisation shrinkage has been an inherent problem in modern composites. This study was designed to establish any significant differences post-gel polymerization shrinkage associated with different light-curing regimens of similar light energy density.

Methods: A light-cure unit (VIP, Bisco) that allowed for independent command over time and intensity was used. The 5 regimens investigated were pulse delay (PD), soft-start (SS); pulse cure (PC), turbo cure (TC) and standard continuous cure (C) [control]. With the exception of TC, light energy density for all curing regimens was fixed at 16 J/cm². A strain-monitoring device and test configuration was used to measure the linear polymerisation shrinkage of 2 mm thick composite specimens (Z100, 3M ESPE) during and post light polymerisation up to 60 minutes. Five samples were made for each curing mode.

Results: Post-gel shrinkage ranged from 0.30% to 0.46% at 60 minutes. The use of PD resulted in significantly lower shrinkage compared to PC, TC, SS

and C. Shrinkage associated with SS was in general significantly lower than C. No significant difference in shrinkage was observed between PC, TC and C at all time intervals.

Conclusion: The use of pulse delay and soft-start regimens significantly decreased post-gel polymerisation shrinkage when compared to standard continuous cure.

I170/SC

Effectiveness of Composite Cure Associated with Different Light-Curing Regimes

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Aim: Effectiveness of composite cure is vital as the physico-mechanical properties of the restoration depend on it. Total light energy rather than light intensity alone influences the polymerisation process. Our study compared the influence of 5 different curing profiles with a standardised light energy density on the effectiveness of composite cure.

Methods: A light-cure unit (VIP, Bisco) that allowed for independent command over time and intensity was used. The 5 regimens investigated were pulse delay (PD), soft-start (SS); pulse cure (PC), turbo cure (TC) and standard continuous cure (C) [control]. Light energy density for all curing regimens was fixed at 16 J/cm². Effectiveness of cure was established by measuring top and bottom Knoop hardness of 2 mm thick composite specimens (Z100, 3M ESPE) using a digital microhardness tester immediately and at 1-day post-polymerisation. Five samples were made for each curing regimen.

Results: Top KHN observed immediately after polymerisation with C was significantly lower than that of PD. At 1 day post-polymerisation, the top KHN obtained with C was significantly lower than that of PD, SS, and T. No significant difference in bottom KHN was observed between the different curing modes immediately after curing. At 1 day post-polymerisation, the bottom KHN obtained with C was significantly lower than that of SS and T.

Conclusion: Effectiveness of cure at the bottom surfaces of composites may be increased by soft-start and turbo polymerisation regimens.

I171/SC

Surgical Management of Non-cirrhotic Portal Hypertension – A Study of 25 Cases

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Aim: Non-Cirrhotic Portal Hypertension (NCPHT) is described as an increase in portal pressure in the absence of cirrhosis. The distinction into cirrhotic and non-cirrhotic varieties is important as the latter carries a better prognosis due to the relatively normal hepatocellular function. Our aim was to find the common causes and presentations of NCPHT, indications for surgery and their results.

Methods: Between 1990 and 1997 we analysed 25 consecutive patients undergoing surgery for NCPHT. All patients underwent an ultrasound, percutaneous liver biopsy and splenoportogram. The modified Child-Pugh grading criteria was used to assess liver function.

Results: Thirteen patients had extra-hepatic portal vein obstruction (EHPVO) with no underlying cause. 11 patients had non-cirrhotic portal fibrosis (NCPF) and 1 had congenital hepatic fibrosis. Upper GI bleed and splenomegaly was present in all but ascites in only 32% of cases. Seventeen patients underwent surgery due to failure of endoscopic sclerotherapy. The remaining 8 patients selected surgery as a 1-time therapy. Fourteen patients underwent a selective portosystemic shunt and the remaining 11 had devascularisation surgery.

Conclusion: Surgery for NCPHT is indicated in cases where sclerotherapy fails or for patient preference for a 1-time procedure. The distal splenorenal shunt is the best procedure if the anatomy is suitable. Devascularisation procedures should be considered only if selective shunts are not feasible. NCPF and EHPVO are by far the 2 most important causes of NCPHT in our setting. Their aetiology remains elusive and is a matter for further research.

I172/SC

Cardiac Time Intervals of Normal Fetuses Using Non-invasive Fetal ElectrocardiographyE CHIA¹, TF HO¹, M RAUFF², W YIP³¹Physiology, Faculty of Medicine, National University of Singapore, Singapore, ²Department of Obstetrics and Gynaecology, National University Hospital, Singapore, ³Department of Paediatrics, Gleneagles Hospital, Singapore**Aim:** To evaluate the various fetal cardiac time intervals from the longitudinal analysis of fetal electrocardiography (fECG) in normal pregnancies. Such normative information will be useful for clinical application in the assessment of fetuses with suspected cardiac diseases or dysrhythmias.**Methods:** Cardiac time intervals are derived from fetal electrocardiograms obtained non-invasively via maternal abdominal electrodes from the 18th week of gestation onwards. The variables measured included the durations of the P wave, PR interval, QRS complex, QT interval and T wave, which were plotted against gestational age.**Results:** Linear regression analysis identified a strong dependency of the durations of P wave, PR interval, QRS complex, QT interval and T wave on fetal gestational age. The mean P wave duration increased from 43.9 ms at 18 to 22 weeks of gestation to 52.9 ms at ≥ 37 weeks ($P < 0.001$). PR intervals were 102.1 ms and 110.1 ms, for fetuses at 18-22 and ≥ 37 weeks ($P < 0.001$), respectively. QRS intervals were 47.4 ms and 51.5 ms ($P = 0.004$), QT intervals were 224.0 ms and 242.7 ms ($P < 0.001$), while T wave durations were 123.8 ms and 152.4 ms ($P < 0.001$), for fetuses at 18-22 and ≥ 37 weeks, respectively.**Conclusion:** This study demonstrates the use of non-invasive fECG monitoring in pregnancy, and provides data on normal fECG cardiac time intervals from 18 to 41 weeks of gestation. These intervals are useful as reference values for the detection of abnormal changes in circumstances where fetal arrhythmias, IUGR and congenital heart defects may occur.

I173/SC

Oral Self-care, Practices and Attitudes of Diabetics in SingaporeHM HTOON¹, LP LIM², CF SUM³, FBK TAY⁴¹Preventive Dentistry, Faculty of Dentistry, National University of Singapore, Singapore, ²Department of Preventive Dentistry, Faculty of Dentistry, National University of Singapore, Singapore, ³Diabetics Department, Alexandra Hospital, Singapore, ⁴Department of Dental Service, Alexandra Hospital, Singapore**Aim:** Diabetes is one of the risk factors in periodontal disease. With the high prevalence of diabetes in Singapore adults, there is no available data on the oral health behaviour of diabetics in the local context. The aim of the study is to find out the oral health attitudes and practices of adult diabetics receiving medical treatment at a diabetic centre**Methods:** A questionnaire survey together with clinical and laboratory parameters were conducted to profile the diabetic patients from a hospital-based Diabetic centre as part of a larger scale study. One hundred and twelve adult diabetics aged 21 to 65 years responded to questionnaires relating to oral self-care, attitudes and practices (15 items) and diabetic conditions (12 items).**Results:** The majority of patients were compliant in their diabetic control. More than two-thirds of diabetics did not visit a dentist annually as well as did not know or were informed that they were at high risk for periodontal disease ($P < 0.05$). Most patients claimed to be brushing regularly (99.1%); interdental cleaning was not practised by a marginal majority (50.5%). Only 9.3% of participants had acceptable level of knowledge on periodontal health ($P < 0.001$) and ability to detect gum disease was also low at 25%**Conclusion:** A preliminary study of diabetics showed that the majority of diabetics have low periodontal health awareness, infrequent dental visits and interdental cleaning behaviour. The findings highlight a need to promote oral health as part of the overall component of medical healthcare for patients with diabetes.

I174/SL

Alveolar Osteoblasts Growth and Function on 3 PCL Scaffolds In VitroYF ZHOU¹, AM CHOU², ZM LI³, V SAE-LIM³, DW HUTMACHER⁴, TM LIM⁵¹Biological Science, National University of Singapore, China, ²Bioengineering, National University of Singapore, Singapore, ³Department Restorative Dentistry, National University Hospital, Singapore, ⁴Department of Orthopaedic Surgery, National University Hospital, Singapore, ⁵Biological science, National University of Singapore, Singapore**Aim:** Most studies on alveolar osteoblasts (AOs) were based on 2-dimensional cultures; little knowledge is available on 3-dimensional cultures. In this study, we investigated AOs proliferation and differentiation on the PCL-TCP (80:20) scaffolds in vitro, mimicking the *in vivo* bone matrix.**Methods:** Proliferations of AOs on the scaffolds were assayed through DNA synthesis rate and the differentiation processes were monitored at both RNA and protein level by RT-PCR and western blots techniques as well as histology.**Results:** AOs finished attachment on scaffolds in 2 hours and, after 28 days; cells evenly distributed and formed multilayers cells and collagen fibers inside the scaffolds. Culture on TCP-PCL scaffolds exhibited significant influence on the expression pattern of AP activity and osteo-related biomarkers osteopontin (OPN), osteonectin as well as collagen type I (Col I) compared to the petri dish culture. OCN, a specific mature osteoblasts marker, expression level of AOs on scaffolds was 30% to 80% higher than that on petri dish. Osteogenic induction (Dex, ascorbic acid and glycerophosphate) down-regulated OCN at both RNA and protein level for AOs in scaffolds while up-regulated OCN for AOs in petri dish. Immunocytochemistry results show that Col I, OPN and OCN signals were strongly detected at the outer part of constructs of AOs-scaffolds and the interface of AOs-scaffolds; while most mineral nodules occurred at the borders of constructs.**Conclusion:** These findings demonstrate that AOs in 3-dimensional scaffolds had different osteogenic characterisation with those on petri dish plates and the 3-dimensional culture system may be a model system for study *in vivo* osteogenesis of alveolar osteoblasts.

I175/SL

Cellular Cardiomyoplasty Using Transgenic Myoblasts Induce Mature Blood Vessel a Better Choice for Cardiac RepairLYE¹, HKH HAIDER², R TAN³, RWGE⁴, IC SONG⁵, PK LAW⁶, EKW SIM⁷¹Department of Cardiac, Thoracic and Vascular Surgery, National University of Singapore, Singapore, ²National University Medical Institute, National University of Singapore, Singapore, ³Department of Cardiology, National Heart Centre, Singapore, ⁴Department of Biological Sciences, National University of Singapore, Singapore, ⁵Department of Experimental Surgery, Singapore General Hospital, Singapore, ⁶Cell Transplants Singapore Pte., Ltd, Singapore, ⁷Department of Cardiac, Thoracic and Vascular Surgery, National University Hospital, Singapore**Aim:** VEGF induces capillary formation. We hypothesise that simultaneous administration VEGF with angiopoietin-1 (Ang-1) using a bicistronic vector together with myoblast transplantation lead to better prognosis for treatment of myocardial infarction.**Methods:** Human skeletal myoblasts carrying Lac-z gene were transduced with adenoviral bicistronic vectors carrying human VEGF165 and Ang-1. Myocardial infarction was created in 17 female pigs by coronary artery ligation and grouped as DMEM injected (group-1 n = 6), Ad-null transduced myoblast transplanted (group-2 n = 5), and myoblast carrying bicistronic vector transplanted (group-3 n = 6). Three weeks later, 5 ml DMEM with or without 3×10^8 cells were intramyocardially injected in and around infarction. Animals were immunosuppressed for 6 weeks using 5 mg/kg/day Cyclosporine-A. Pigs were euthanised and hearts were explanted at 6-weeks post-treatment and processed for histological studies.**Results:** Transduced myoblasts efficiently secreted VEGF165 and Ang-1 simultaneously as revealed by dual fluorescent immunostaining and RT-

PCR. Extensive survival of the Lac-z positive myoblasts was observed in pig heart. Average vascular density at low power field (x100) by double immunofluorescent staining for vWFactor-VIII and smooth muscle actin in group-3 (45.2 ± 5.87 ; 38.6 ± 2.13) was significantly higher than group-1 (16.18 ± 0.91 $P < 0.05$; 7.88 ± 0.52 $P < 0.05$) and group-2 (26.57 ± 2.09 $P < 0.05$; 20.14 ± 1.68 $P < 0.05$) at 6-weeks post-treatment. Mature blood vessel count and regional blood flow in group-3 was the highest (86.33% $P < 0.05$; 2.91 mL/min/g $P < 0.05$). Significant improved EF in group-3 (50.5% $P < 0.05$) was achieved compared with DMEM group.

Conclusion: Simultaneous VEGF165 and Ang-1 gene delivery using skeletal myoblasts as carriers lead to angiomyogenesis with higher percentage of mature blood vessel formation with better prognosis.

I176/SL

Antithrombin III and D-dimer Levels and Their Association with Survival Outcome at 36 Months from Ovarian Cancer

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Aim: To determine the association between pre-operative haemostatic levels of Antithrombin III (ATIII) and D-dimer with survival outcome at 36 months from ovarian cancer. The association of fibrinolytic system and cancer especially in primary tumour extracts to disease-free interval, tumour growth and angiogenesis have been reported. Similarly, systemic enhanced fibrinolysis and thrombin generation are useful markers of disease state.

Methods: The haemostatic data for pre-operative levels of ATIII and D-dimer from 33 patients with ovarian cancer (FIGO Stage I/II n = 10), Stage III/IV n = 23) and aged between 16 years and 80 years (mean 52 ± 14.1 years) were analysed for their association for disease-outcome at 12 months, 24 months and 36 months.

Results: The mortality rate within 36 months was 23 patients and the remaining 10 patients (Stage I/II n = 8) were still living. The median ATIII level for those still living was 104.5% and D-dimer 1940 ng/mL. The median ATIII level from mortality was 88% and D-dimer 9688 ng/mL. There were significant associations ($P < 0.01$) with survival outcome at 12 months (mortality n = 12), 24 months (mortality n=18) and 36 months (mortality n=23) with both ATIII and D-dimer levels.

Conclusion: Mortality from ovarian cancer was associated with further enhanced thrombin generation together with fibrinolysis compared to those still living past 36 months from disease. It is therefore suggested that ATIII, together with D-dimer levels, be used as prognostic haemostatic markers for disease outcome especially in patients with advanced ovarian cancer.

I177/SL

Detection of Epsilon-globin-positive Fetal Primitive Erythroblasts in Newborns with Trisomy 18 (Edward Syndrome)

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Aim: The epsilon-to-gamma globin switch is completed in normal fetuses by 14 weeks gestation. Fetal erythroblasts are increased in cord blood in pregnancies complicated by fetal aneuploidy, but it is not known if the globin switch is delayed. We hypothesised that the epsilon-to-gamma globin switch is not complete in pregnancies complicated by trisomy 18, and that these neonates have epsilon-globin-positive primitive erythroblasts up to term.

Methods: Cord blood from 2 term neonates diagnosed antenatally to have trisomy 18, and from 20 normal neonates was processed using magnetically-activated cell sorting to obtain glycophorin-A-(GPA)-positive fetal erythroblasts. Morphology of these cells was evaluated by Wright stain, and the presence of epsilon-globin within the cytoplasm was determined using alkaline phosphatase immunohistochemistry. Chromosome aneuploidy was confirmed with fluorescence *in situ* hybridisation (FISH). The Mann-Whitney test was used to compare 2 independent samples with non-parametric distribution.

Results: Typical fetal primitive erythroblasts were identified only in the cord

blood of affected neonates. These cells were 42-fold higher in neonates with trisomy 18 compared with normals (1038 vs 25). In both cases of trisomy 18, primitive erythroblasts were epsilon-globin positive (100%; $P < 0.001$).

Conclusion: It is rare for pregnancies known to be complicated with trisomy 18 to continue to term. This is the first report of epsilon-globin-positive fetal primitive erythroblasts identified at such a late gestation (at term). This observation is important: it suggests that either chromosome 18, or the presence of an additional autosome, has a role in the globin-switching mechanism. Understanding and control of the globin switch will expand the therapeutic options available for the management of thalassaemia.

I178/SL

Insulin Sensitiser Ligands for Peroxisome Proliferator-Activated Receptor Gamma Inhibit Proliferation of Human Uterine Leiomyoma Cells

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Aim: Uterine leiomyomas (fibroids) are the most frequently occurring tumour of the female reproductive tract and clinically affect approximately 30% of childbearing-aged women. Effective treatment options are few, and fibroids are the primary cause of hysterectomies in women worldwide. In search for alternative treatments, we determined the efficacy of novel phyto-biomolecules to inhibit or modulate the proliferation of patient-derived myometrial and fibroid cells.

Methods: Primary cell cultures of myometrial and fibroid smooth muscle cells were established from patients undergoing hysterectomy for uterine fibroids at the National University Hospital, Singapore. Cells were characterised by immuno-confocal microscopy and a colorimetric cell proliferation assay. Expression of growth regulatory proteins were determined by immuno-blotting.

Results: Myometrial primary cell cultures exhibited a higher proliferative index compared to their matched fibroid cells. A phyto-biomolecule compound that activates the Peroxisome Proliferator-Activated Receptor (PPAR) gamma showed growth inhibitory effects on uterine cells. Furthermore, the thiazolidinedione PPAR gamma ligand Pioglitazone showed significant dose-dependent inhibition of uterine cell proliferation and cell numbers. The anti-proliferative activity of Pioglitazone was determined within the fibroid patient population with an initial sample size of five patient-derived fibroid cell cultures. The combined results showed a statistically significant reduction of cell proliferation. Fibroid cells were more sensitive to Pioglitazone growth inhibition than their normal counterparts. Growth inhibition was independent of cell cycle inhibition and apoptosis.

Conclusion: Our results suggest the possibility of using PPAR gamma ligands as non-surgical therapeutic agents for the treatment of uterine fibroids. The patient-derived cell cultures provide a useful system to reveal potential targets for intervention of this common gynaecological malady.

I179/SL

Lack of Association of the Missense Glu298Asp Variant of the Endothelial Nitric Oxide Synthase Gene with Preeclampsia in a Malay Population

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Aim: Since nitric oxide (NO) synthesised by endothelial NO synthase (eNOS/NOS3) is thought to be crucial for maintaining maternal systemic vasodilation and reducing vascular reactivity during normal pregnancy, several studies have focused on its possible role in preeclampsia, with mixed results. It is thought that the NOS3 gene has a variable role depending on the population of origin. We tested for evidence of association between the chromosome 7q36 located NOS3 gene and preeclampsia in the local Malay population.

Methods: In a preliminary study, 39 preeclamptic Malay mothers and 54

non-hypertensive Malay mothers were recruited. Blood samples were collected from mothers and matched umbilical cords after delivery, and DNA was extracted using standard procedures. Exon 7 of the NOS3 gene was amplified by PCR, and sequenced to determine the SNP genotype at nucleotide position 894 (G/T). Allele frequencies were compared between cases (with preeclampsia) and controls, as well as between offspring of cases and offspring of controls, using the Chi Square Test.

Results: The genotype frequencies in preeclamptics and controls were in Hardy-Weinberg equilibrium. There was no significant difference in either genotype or allele frequencies between preeclamptic ($T = 0.19$) and non-preeclamptic ($T = 0.20$) mothers, or between offspring of preeclamptic ($T = 0.14$) and non-preeclamptic ($T = 0.19$) mothers.

Conclusion: The SNP genotyping results show no evidence of association between either allele of the eNOS/NOS3 Glu298Asp polymorphism and preeclampsia in our preliminary cohort of patients. Therefore, this gene may not be an important contributor to the etiology of preeclampsia in the local Malay population.

I180/SL

Enrichment of Epsilon-globin-positive First Trimester Fetal Primitive Erythroblasts from Maternal Circulation: Novel Protocol for Early Non-invasive Prenatal Diagnosis

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Aim: Prenatal diagnosis using fetal erythroblasts enriched from maternal blood is an ideal non-invasive strategy, and eliminates the risk of amniocentesis. Only 1 in 10 million maternal nucleated cells are fetal (1 cell/ml maternal blood), and poor enrichment limits the application of this technology to clinical practice. We hypothesised that a multi-step strategy could be developed to enrich these rare cells from maternal blood.

Methods: Density gradient centrifugation, Magnetically Activated Cell Sorting (MACS), selective anucleate erythrocyte lysis, fluorescence immunocytochemistry and chromosomal fluorescence *in situ* hybridisation (cFISH) were combined to develop a multi-step enrichment strategy.

Results: Protocol developed: Maternal whole blood diluted in phosphate-buffered saline was centrifuged over Percoll 1118. Mononuclear cells were recovered from the gradient density interface. White blood cells were depleted using anti-CD45 antibodies, and red cells were selected using anti-GPA antibodies by MACS. Fetal primitive erythroblasts were separated from maternal anucleate erythrocytes by selective lysis with ammonium chloride/1mM acetazolamide mixture, and unlysed cells cytospun onto plain glass slides. Epsilon-globin-positive fetal primitive erythroblasts were identified by a novel combined fluorescence immunocytochemistry and cFISH technique. Protocol tested: This new protocol was tested by sorting for, and identifying, fetal primitive erythroblasts from 20 ml maternal blood obtained after surgical termination of pregnancy. We successfully enriched 17 fetal cells from 20 mL maternal blood ($n = 3$ samples). In all cases the fetal gender predicted matched cFISH results on control trophoblast tissue.

Conclusion: Our novel protocol successfully enriched epsilon-globin-positive fetal primitive erythroblasts from first trimester maternal blood. It is a promising tool for early non-invasive prenatal diagnosis.

I181/SL

The Development of a Conjunctival Epithelial Equivalent with Improved Proliferative Properties Using a Multistep Serum-Free Culture System

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Aim: The ability of bioengineered tissue-equivalents to remain proliferative *in vivo* is critical for promoting tissue regeneration. We investigated the use of a multistep serum-free culture system in developing a conjunctival

equivalent with improved *in vitro* and *in vivo* proliferative properties, and compared it to conventional serum-supplemented conditions.

Methods: Conjunctival epithelial cells were cultivated on human amniotic membrane using a multistep serum-free culture system, under submerged and air-lifted conditions. The Bromodeoxyuridine (BrdU) ELISA proliferation assay, colony-forming efficiency (CFE), and number of cell generations achieved were compared with serum-containing media. The *in vivo* proliferative capability of the tissue-constructs were evaluated by xenotransplantation onto severe combined immune-deficient mice. Cultured cells were evaluated for the expression of keratins 4, 19, 3, and MUC5AC goblet cell mucin.

Results: Epithelial cells cultivated in serum-free media (BrdU absorbance, 1.91 ± 0.08 ; cell generations, 25.6 ± 4.5) were more proliferative than those cultivated in serum-containing media (BrdU absorbance, 1.06 ± 0.08 ; cell generations, 12.1 ± 3.0). The serum-free derived epithelial equivalents demonstrated a significant increase in proliferation and stratification following transplantation. Cells that were air-lifted for 6 and 12 days had a reduced proliferative capacity *in vitro* and *in vivo* compared to submerged cultures. Cultured cells expressed keratins 4 and 19, and MUC5AC. Electron microscopy demonstrated a basal lamina with numerous hemidesmosomes.

Conclusion: We describe the novel use of a multistep serum-free culture system in developing a conjunctival tissue-equivalent with improved proliferative and structural properties, which are crucial for enhancing graft-take and regeneration of the conjunctival surface following clinical transplantation.

I182/SL

Enhancement of Tendon Allograft Osteointegration Using Mesenchymal Stem Cells in a Rabbit Model of Anterior Cruciate Ligament Reconstruction

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Aim: Soft-tissue allografts provide an excellent alternative for revision procedures where suitable autogenous tissues have been previously compromised. However the use of allografts is controversial due to a higher failure rate and slower biological incorporation. Mesenchymal stem cells (MSCs) are currently under investigation as potential agents to enhance bony and cartilage healing in a variety of orthopaedic disorders. We hypothesised that the application of MSCs at the tendon bone junction during ACL reconstruction might result in both acceleration and enhancement of osteointegration, hence improving the success rate of ACL reconstruction with allografts.

Methods: Bilateral ACL reconstructions with tendoachilles allografts were performed in 18 adult rabbits. On 1 limb the graft was coated with allogeneous MSCs in a fibrin glue carrier, while the contralateral limb served as control with no application of MSCs. The reconstructions were assessed histologically and biomechanically at 2, 4 and 8 weeks.

Results: Histologic analysis of the controls revealed granulation tissue and fibroblasts with the development of some collagen fibres resembling Sharpey's fibres by 8 weeks. The tendon bone interfaces of the MSC enhanced ACL reconstructions, however, consistently demonstrated large areas of fibrocartilage between tendon and bone as early as 4 weeks. On biomechanical testing, the MSC enhanced grafts had significantly higher load-to-failure rates than did controls at all time frames.

Conclusion: Use of MSCs to enhance allograft osteointegration is a novel method offering the potential of more physiological, earlier healing and biomechanically stronger allograft ligament reconstructions, and provides an effective ACL reconstruction alternative, for both primary reconstruction and for revision surgery.

I183/SL

Enhancement of Meniscal Regeneration Using Mesenchymal Stem Cells in a Porcine Model

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Aim: To investigate the gross and microscopic morphologic and biomechanical effects of the administration of thrombin glue and mesenchymal stem cells in the enhancement of surgical repair of radial meniscal tears in the porcine model.

Methods: The pig model of a radial meniscal tear is created by a surgical cut made in a radial direction at the inner two-thirds of the medial meniscus. Three groups of animals are studied. For the control, the cut is not treated. The second group has the meniscal tear treated with thrombin glue and suture repair. The third group has the meniscal tear treated with thrombin glue with mesenchymal stem cells and surgical repair. The mesenchymal stem cells are harvested from each individual pig and processed by the technique developed by the principle investigator. The animals are sacrificed at 8 weeks and the menisci undergo the following investigations: Determination of meniscal vascular anatomy by histology Gross and microscopic examination of the repair process.

Results: Imaging of the porcine meniscus revealed relative avascularity of the inner two-thirds of the meniscus compared to the outer third. This is similar to that seen in the human model. Healing was seen in the 2 treated groups on histologic examination. The group treated with mesenchymal stem cells showed significantly improved healing compared to the group not treated with stem cells.

Conclusion: The result of this basic science study validates the safety and efficacy of using mesenchymal stem cells to enhance the healing process of central meniscal tears and justifies further investigation of this treatment in clinical trials.

I184/SL

Temperature and Temporal Effect on Viability and Proliferation of Bone Marrow Stem Cells

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Aim: In cell transplantation studies there is a time lag between cell preparation and implantation. In this critical period, cells are out of their physiological conditions. Our study investigates bone marrow stem cell (bMSC) viability and colony forming efficiency (CFE) at varying temperatures over time.

Methods: Cells were removed from culture and washed in PBS. They were subjected to varying temperatures of 4, 22 and 37°C for periods of 1, 3 and 5 hours. Cell viability was assessed by Trypan blue exclusion and fluorescent labelling. CFE was assessed by seeding cells low densities and counting colonies that form.

Results: Viability at 4°C remained above 90% for up to 5 hours. At 22°C and 37°C, viability decreased from 91% to 80% and 86% to 49% from 1 hour to 5 hours respectively. CFE at 5 hours post incubation showed that at days 15 and 30, the control group had significantly more colonies than the test groups. Although cells remain more viable at 4°C, the CFE for both 4°C and 22°C had no significant difference and were much lower than the control. 37°C appears to be the least favourable temperature to store cell preparations.

Conclusion: Delay in implantation, despite preservation in ice, may have significant adverse effects on cell viability and ability to proliferate.

I185/SL

Selection of Cell Source for Ligament Tissue Engineering

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Aim: Anterior cruciate ligament (ACL) fibroblasts, medial collateral ligament (MCL) fibroblasts, mesenchymal stem cells (MSC) and embryonic stem cells (ESC) are potential cell sources for ligament tissue engineering. Research on ESC is currently associated with technical difficulties, long-term uncertainty of outcome and ethical considerations. Therefore, the use of lineage specific cells and adult stem cells are preferred in tissue engineering applications. The aim of the current study is to determine the most suitable cell source for potential ligament tissue engineering.

Methods: Cell proliferation and collagen expression of ACL fibroblasts, MCL fibroblasts and MSC were examined. Collagen type I, II and III, and alpha smooth muscle actin of these cells were evaluated using immunohistochemistry techniques. Green Fluorescent Protein (GFP)-transfection technique was used to trace the MSC in the tissue engineered ACL construct.

Results: The results revealed that MSC had better proliferation rate and total collagen expression than ACL and MCL fibroblasts. In the mean time, MSC, ACL and MCL fibroblasts were all strongly stained with antibodies of collagen type I and III, and alpha smooth muscle actin while negatively stained with collagen type II antibody. The loaded MSC could be found survival even at 6 weeks after implantation.

Conclusion: The results showed that MSC are a better cell source than ACL and MCL fibroblasts for anterior cruciate ligament tissue engineering.

I186/SL

Recovery in the Expression of Myosin-Heavy-Chain Isoforms, 7 Months after Repairing a Completely Lacerated Skeletal Muscle with Microanastomosis of the Intramuscular Nerve Branch

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Aim: The Myosin heavy chain (MHC) isoforms are proteins associated with muscle fibre contraction, and play a predominant role in regulating the contractile characteristics of a fibre. In the repair of injured muscles, the recovery of MHC expression would suggest a return of its contractile characteristics. This study investigates the expression of the MHC isoforms in lacerated muscles that were repaired, looking at the effects of repairing the intramuscular (IM) nerve branch on the contractile characteristics of the muscle.

Methods: Completely lacerated medial gastrocnemius (MG) muscles in adult rabbits were used in 3 different Repair Groups: A) No muscle repair; B) Immediate muscle repair and C) microanastomosis of the main IM nerve branch, followed by immediate muscle repair. A control muscle repair model (Group-D) involved one that was lacerated but with the IM preserved intact. The contralateral muscles serve as controls. Assessment was at 5 months and 7 months, with the isometric contractile properties measured.

Results: Full recovery of MHC I and MHC II in the distal segment for Group-C, and a near full recovery for Group-D were observed at 7 months. While Group-A and -B had an adverse reduction in MHC II and drastic increase in MHC I reflect an in-balance probably due to minimal reinnervation in some segments.

Conclusion: The data suggest that IM nerve repair with muscle repair will play an important role in returning the contractile properties of the muscle.

I187/SL

Functional Evaluation of Patients with Anterior Cruciate Ligament Deficiency

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Aim: Currently, functional assessment following anterior cruciate ligament (ACL) injury relies mainly on clinical examination and passive testing of knee laxity. It was hypothesised that direct measurement of ambulatory function and more demanding activities such as stair climbing would be an appropriate method to perform functional evaluation of the ACL.

Methods: Six healthy volunteers and 9 patients with isolated ACL deficiency participated in this controlled study. Gait analysis was performed during stair climbing using a 5-camera motion capture system and force platform fitted into a custom staircase. The data were analysed to study between-limb differences for each subject.

Results: All subjects walked up and down the stairs without pain or discomfort at the same speed of 0.6 m/s. From gait analysis data, patients with ACL deficiency displayed a significant ($P < 0.05$) reduction of up to 50% in

peak knee flexion moments in their involved knee during stair ascent. Peak moments in the involved knees reached only 0.3 Nm/kg. During stair descent, at over 60%, the reduction of knee moments in the involved knee was more pronounced. Moments in the involved knees reached only 0.2 Nm/kg. For the control group there were no significant differences in knee moments between left and right knees. There were also no significant differences between the uninvolved knee of patients and the knees of the normal control group.

Conclusion: Direct measurement of ambulatory function is feasible and a practical tool for guidance of the management and rehabilitation process for ACL deficiency.

I188/SL

Differential Expression of Cell Cycle Regulator Proteins in Nasal Polyposis

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Aim: We present an immunohistochemical profile of cell cycle regulator proteins in diseased epithelium of nasal polyps. Nasal polyposis affects 2% to 4% of the population. Precise mechanisms contributing to its pathogenesis are not clearly understood. Recurrent infections, rupture of the epithelium and production of granulation tissue, inhaled or food allergens, T cell disturbances, and aerodynamic factors have all been implicated. Its recurrent nature suggests aberrations of cell cycle regulation of diseased nasal epithelium, contributing to increased epithelial proliferation.

Methods: Pathology specimens of 28 patients with unilateral/bilateral nasal polyposis, who underwent functional endoscopic sinus surgery were obtained. Pathology specimens of inferior turbinates of 10 patients who underwent unrelated surgery were used as normal controls. A standardised immunohistochemical protocol for staining of cell cycle regulator proteins (p21, p27, p53, p63 and Ki67) was applied to both set of specimens.

Results: There is differential expression of p21 and p27 between epithelium of nasal polyps and inferior turbinate mucosa. Marked reduction in expression of p27 is noted. This is often present in conjunction with a higher proliferation index (as indicated by Ki67 expression). We report no difference in p53 and p63 expression in both types of tissue, in contrast to reports by previous authors.

Conclusion: Dysregulation of epithelial proliferation is present in nasal polyps. There is marked reduction in p21 and p27, both of which are suppressors of the cell cycle in normal, proliferating cells.

I189/SL

Bcl-x is Co-expressed with GFAP in Reactive Gliosis and Various Gliomas: A Potential Marker for Diagnostic Pathology

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Aim: Bcl-x is an anti-apoptotic molecule that has been noted to stain reactive astrocytes. We set out to study the expression of bclx and GFAP in various gliomas and reactive brain conditions in an attempt to discover if bcl-x might be useful as a second marker for gliomas.

Methods: Cases of gliomas and reactive brain disorders were retrieved and the histology reviewed. These included astrocytomas (pilocytic, diffuse fibrillary, anaplastic, SEGA), ependymoma, ganglion cell tumour, GBM, oligodendroglioma, mixed tumours (oligo-astrocytomas, ganglioglioma) and subependymoma. Cases of reactive gliosis, cerebritis and mesial temporal sclerosis were also included. Immunostaining for bcl-x and GFAP was performed, following heat-induced antigen retrieval and using DAB as chromogen. Selected cases were also subjected to double immunofluorescent labelling using both GFAP and Bcl-x.

Results: Expression of bcl-x closely follows that of GFAP with strong expression in both reactive astrocytes and astrocytomas. There is more focal expression in neoplastic cells of other gliomas. Immunostaining for bcl-x is

generally more distinct with less background, compared to that for GFAP. Expression of both GFAP and bcl-x is more focal in oligodendrogliomas, with staining of mainly intervening astrocytic processes. Double immunolabelling confirms the co-expression of bcl-x and GFAP.

Conclusion: Bcl-x is co-expressed with GFAP in various gliomas and reactive brain conditions. Immunostaining for bcl-x is generally more distinct and intense. In the appropriate context, immunostaining for bcl-x may be useful in the identification of gliomas, in particular, astrocytomas.

I190/SL

The Role of Activin System in Keloid Pathogenesis

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Aim: Activin A is a dimeric protein and a member of the TGF- β superfamily. It regulates various aspects of cell growth and differentiation in many tissues. Activin signalling is modulated by a variety of extracellular modulators like inhibin and follistatin, acting at different steps. Our aim is to investigate the biological response of normal (NF) and keloid fibroblasts (KF) on activin A and follistatin stimulation.

Methods: Different strains of NF and KF were seeded at 50% confluence in DMEM/10% FCS and then starved in plain DMEM for 48 hours. Activin A (Concentration of 50, 100 ng/mL of commercial activin A) and follistatin were added to cells. Synergistic effect of activin with TGF- β 1 was explored by treating cells with TGF- β 1 along with activin. The production of collagen, fibronectin, α -SMA and PCNA by NF and KF under activin A and follistatin stimulation was assessed by Western blot using antibodies against these proteins.

Results: Normal and keloid fibroblasts showed an increased expression of collagen when treated with activin A. No significant difference was observed in the production of collagen in activin, TGF- β and activin + TGF- β treated cells suggesting lack of synergism between the two. Interestingly, though follistatin seemed to stimulate the production of collagen, when added with activin A completely stopped its expression. A similar expression profile was seen for fibronectin, α -SMA and PCNA. However, expression of these was seen in follistatin + Activin treated cells unlike the lack of expression of collagen observed upon similar treatment.

Conclusion: The expression profiles of the key growth factors indicate a possible role of activin A and follistatin in keloid pathogenesis.

I191/SL

Epithelial Interactions in Keloids Induce Contraction of Fibroblast Collagen Lattices

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Aim: Keloid scars represent a pathological response to cutaneous injury. The pathological biology of these scars remains poorly understood. The role of epithelial-mesenchymal interactions in keloid pathogenesis and scar contracture has recently been explored. Our aim is to explore the role of epithelial-mesenchymal interactions in scar contracture and keloid pathogenesis.

Methods: A co-culture model was employed wherein keloid and normal keratinocytes were co-cultured with keloid or normal fibroblasts, and the conditioned media from day 5 co-cultures collected to study the effect of the paracrine secretions on contraction of an in vitro fibroblast-populated collagen lattice model (FPCL). Anti-TGF-beta1 neutralising antibody was added to the conditioned media to see its effect on FPCL. In addition, keloid and normal fibroblasts were cultured on chamber slides and treated with conditioned media from co-culture and non co-culture series. Immunohistochemical analysis was done to investigate the expression of alpha-smooth muscle actin.

Results: 1) Keloid keratinocyte/keloid fibroblast co-culture conditioned media brought about increased contraction of the collagen lattice as compared to non co-cultured conditioned media. In addition increased lattice contraction was induced in keloid FPCL, compared to lattices populated with normal fibroblasts. 2) Expression of alpha-smooth muscle actin was increased in keloid derived fibroblasts treated with keloid keratinocyte/keloid fibroblast-

co-culture conditioned media. 3) Anti-TGF-beta 1 neutralizing antibody attenuated contraction of keloid FPCL'S when added with the co-culture conditioned media.

Conclusion: Paracrine output from epithelial-mesenchymal interactions between keloid keratinocytes and keloid fibroblasts modulate contraction of fibroblast-populated collagen lattices, strongly suggesting its role in scar and wound contraction. Our work also underscores the role of TGF-beta 1 as a key player in keloid pathogenesis

I192/SL

Specific Target Sp1 Transcription Factor – A New Therapeutic Approach for Keloids

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Aim: Accumulation of extracellular matrix (ECM) components is a direct cause of fibrotic conditions resulting in keloid scars. Several ECM promoters are regulated by Sp1. Bisanthracycline WP631 and Mitoxantrone are potent inhibitors of Sp1-activated transcription. They competitively bind for the common Sp1 binding site, thereby directly preventing the transcription of ECM genes. Our aim is to investigate the effects of WP631 and Mitoxantrone on collagen, fibronectin and alpha-smooth muscle actin (SMA) expression.

Methods: A co-culture model was used whereby keloid and normal keratinocyte were co-cultured with keloid and normal fibroblast. Normal, keloid and hypertrophic scar fibroblasts were also cultured independently. Additionally, a reverse inhibitory action of the drugs was studied to detect the expression of ECM genes from an inhibitor suppressed condition back to a non-inhibitor condition with removal of the drug after 24 hours. Cultured cells were exposed to both drugs for 24 and 36 hours. Cell lysates were assayed.

Results: WP631 as compared to Mitoxantrone was able to suppress expression of collagen and fibronectin more effectively in both 24 and 36 hours. However, immuno-detection on alpha-SMA yielded almost consistent results showing comparatively same band intensity from the positive control, suggesting that the alpha-SMA gene might not have a binding site for Sp1 at its promoter. Thus a different transcription factor might be required for the transcription of alpha-SMA gene. Efforts to detect Sp1 level revealed no significant differences.

Conclusion: These preliminary data suggest that WP631 and Mitoxantrone could be used in the treatment of fibrotic diseases such as keloid.

I193/SL

Autologous Myoblasts Transduced with a Novel Bicistronic Adenoviral Vector Expressing VEGF and Angiopoietin-1 in a Rabbit Model of Hind Limb Ischaemia

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Aim: The role of VEGF and Angiopoietin-1 (Ang-1) in therapeutic angiogenesis has been well documented. We aim to assess the efficacy of autologous myoblasts as carriers of VEGF165 and Ang-1 in improving circulation in a rabbit model of hind limb ischaemia.

Methods: Primary rabbit myoblasts were isolated and expanded in culture. They were labelled with retrovirally-transduced lac-z gene, 4, 6-diamidino-2-phenylindole (DAPI) and 5-bromo-2'-deoxy-uridine (BrdU). Adenoviral bicistronic vector (Ad-Bic) expressing for VEGF165 and Ang-1 genes was used for myoblasts transduction. Hind limb ischaemia model was created in New Zealand white female rabbits by femoral artery ligation and assessed by angiography. The animals were grouped to receive intramuscularly myoblasts transduced with Ad-Bic group-1 n=10, non-transduced myoblasts group-2 n = 10 and DMEM without myoblasts group-3 n = 8. Six weeks post treatment, neovascularisation in the limb was assessed by angiography. The animals

were euthanised and harvested for histological studies.

Results: The skeletal muscle biopsies generated >75% to 80% desmin positive myoblasts. In vitro transduction efficiencies of lac-z and Ad-Bicis were 80% and 75-80% respectively. Extensive myoblasts survival was observed in groups-1 and 2 as visualised by DAPI, BrdU and lac-z staining. Angiography revealed enhanced collaterals in group-1 as compared to groups-2 and 3. Immunostaining for vWFactor-VIII confirmed increased capillary density in group-1 (19.04 ± 1.59) compared to group-2 (5.69 ± 0.51) and group-3 (3.03 ± 0.20).

Conclusion: Therapeutic angiogenesis using myoblast-mediated synergy between Ang-1 and VEGF provides a potential therapeutic option for treatment of peripheral vascular disease.

I194/SL

The Effect of Chronic Bladder Outlet Obstruction on the Expression of Neuronal Nitric Oxide Synthase in the Guinea Pig Urinary Bladder

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Aim: To examine the effect of chronic partial outlet obstruction on expression of neuronal nitric oxide synthase (nNOS) in the intramural ganglion cells of the guinea pig bladder.

Methods: Partial urethral ligation was done in 17 young male guinea pigs. In addition, 3 served as controls while 7 had sham operation. The animals were sacrificed at 2, 4, 6, 8 and 12 weeks and nNOS immunohistochemistry performed on the intramural neurons of the bladder. nNOS positive ganglia were individually counted and plotted against duration of obstruction. In addition, mRNA expression of nNOS was done using qRT-PCR technique.

Results: All the guinea pigs displayed signs of bladder outlet obstruction. At 2 weeks post-ligation, the number of nNOS positive neurons declined to 2366 ± 260.97 compared to 3403 ± 604.70 in normal controls ($P < 0.05$). The decrease was most drastic at 4 weeks (1342 ± 143.13) ($P < 0.01$ compared to control). Many neurons underwent degenerative changes. At 6 weeks, the numbers rose from its 4 weeks nadir to levels near 12-week shams. qRT-PCR showed 42.4% down-regulation of nNOS expression at 4-weeks followed by 75.8% increase by 12-weeks.

Conclusion: We have successfully created the animal model of chronic bladder outlet obstruction to simulate gradual obstruction seen in BPH in humans. A decline in nNOS-positive neuron numbers followed by compensatory increase towards near-normal levels by the third month suggest attempts to up-regulate nitric oxide bioactivity following bladder outlet resistance.

I195/SL

Biodegradable Stent – A Safe and Effective Stent for the New Century

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Aim: To make use of the biodegradable material to produce a stent that is inert and degradable within a defined period without causing obstruction, infection and encrustation. The stent will have a variable time span predetermined to avoid distress and discomfort to the patients.

Methods: Biodegradable stent would be radiological opaque with bromium sulphate. The study was carried out for 6 months. Six dogs were used for this study with 6 right ureters inserted with the normal stents and the other side inserted with the biodegradable stents. Postoperatively, the dogs were monitored for general well-being with urine output and infection. Regular X-rays were taken at monthly intervals to determine the status of the stent. The dogs were sacrificed 3 months later and the ureters were taken out. Histological evaluation was performed.

Results: All 6 dogs underwent surgery uneventfully. Among the 6 dogs, only 1 dog developed complications. The biodegradable side had been normal and the stent degraded within the time without trace. Histological analysis showed no significant changes on the ureters both at the site of the anastomosis

and 4 cm from it.

Conclusion: With this preliminary evaluation of the biodegradable stent, it is promising that the biodegradable stent is able to perform the same function as the commercial stent without causing obstruction, impaction and other complications. The future use of the biodegradable stent can be further evaluated with the incorporation of medication. This will further add value and function of the stent.

1196/SL

Fracture Toughness of Resin-based Dental Composite Restoratives

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Aim: This research was aimed to determine the fracture resistance of dental composite restoratives using the single-edge notch (SEN) specimens.

Methods: The materials investigated were nanofill (Filtek Supreme [FS], 3M), minifill (Z100 and Filtek Z250, 3M), microfill (A110, 3M), flowable (Filtek Flow [FF], 3M), poly-acid modified (F2000, 3M; Dyract Xtra [DX], Dentsply), giomer (Beautifill [BF], Shofu) and ormocer (Admira [AV], Voco) composites. Five specimens (25 x 2 x 2 mm³) of each material were prepared and conditioned in distilled water for 1 week at 37°C. A sharp notch was then formed on the middle of the specimens using a diamond saw and a razor blade was used to initiate the precrack producing a precrack length to thickness (a/w) ratio of about 0.5. The specimens were conditioned in distilled water at 37°C for another 1 week prior to the fracture test. The 3-point bending test was conducted using a universal testing system at a crosshead speed of 0.5 mm/min. Inter-material fracture toughness was compared with the use of ANOVA/post-hoc Scheffe's tests at a significance level of 0.05.

Results: The fracture toughness (K1c) ranged from 0.51 to 1.19 MPa.m^{0.5}. The K1c of Z100 and FS was significantly higher than DX, AV, F2000 and A110. Z250 was significantly more fracture resistant than F2000 and A110. The microfill A110 was found to have the lowest toughness.

Conclusion: The fracture toughness of dental composite restoratives was material dependent. Dental composites which were reinforced by zirconia and silica glass filler particles may have higher resistance to fracture.

1197/SL

Physical Properties of a New Root-end Filling Material

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Aim: The physical properties of a new root-end filling material we developed were studied and compared to ProRoot MTA (Tooth Coloured Formula) (WMTA).

Methods: The new material consists of a powder and a liquid. A mixture of both components resulted in a material with a handling characteristic and consistency similar to commercially available root-end filling materials such as IRM and Super EBA. The physical properties of this new material as well as WMTA were evaluated according to the ISO standards.

Results: The radiopacity of the new material was equivalent to 4.5 mm of aluminium while that of WMTA was 6.5 mm of aluminium. The pH of the new material was 11.4 when freshly mixed, rising to 12.7 after 60 minutes, while that of WMTA was 11.4 when freshly mixed, rising to 13.0 after 60 minutes. The initial and final setting times of the new material were 40 minutes and 140 minutes respectively, while that of WMTA was 45 minutes and 145 minutes respectively. The solubility of the new material was 2.14% mass fraction, while that of WMTA was 1.28% mass fraction at 24 hours. The results showed that the new root-end filling material had physical properties comparable to WMTA, as well as satisfying the requirements for use as a dental root canal sealing material.

Conclusion: Further development of this material is indicated to produce a root-end filling material which combines the superior biocompatibility of WMTA with the excellent handling characteristic of materials such as IRM and Super EBA.

1198/SL

Evaluation of the Feasibility and Safety of Ultra Polycaprolactone as a Dural Substitute

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Aim: Dural grafts are required to cover defects resulting from excision or shrinkage following neurosurgical procedures. Such grafts will need to possess suitable mechanical properties and be non-immunogenic. It is also imperative that they be easily manufactured and easy to handle during surgery. Polycaprolactone (PCL), a biodegradable polymer has been used as a suture material. Recently, it was found that biaxial drawing of PCL into ultra-thin films produces a membrane-like sheet that could possibly be used as a dura substitute. The aim of this study was to evaluate the feasibility and safety of ultra-thin PCL film as a dura substitute.

Methods: PCL pellets were dissolved in methylene chloride and cast over glass sheets forming films. These films were biaxially drawn with a machine into ultra-thin sheets. Under general anaesthesia, 15 rabbits had mid-cranial incisions made. Skin flaps were raised and 2 9-mm circular defects were created on the cranium, exposing the dura. Dura defects of similar sizes were made. The left defect was covered with ultra-thin PCL film. The right was replaced with excised dura as a control. The rabbits were observed and the dural implants were inspected at 6 and 12 weeks before sacrifice.

Results: The process of manufacturing the films was simple. It was easy to handle during surgery. No complications like infections etc. were noted in the rabbits.

Conclusion: PCL is safe as a dura substitute. It is readily available and easy to handle during surgery. We wish to acknowledge: NMRC/0459/2000 and NMRC/0747/2003.

1199/SL

Transplantation of Bone Marrow Derived Stem Cells for Cardiac Repair

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Aim: The present study assesses cellular angiogenesis using bone marrow derived stem cells (BMCs) for the treatment of myocardial infarction.

Methods: Myocardial infarction was created in adult female New Zealand white rabbits (n=15) by LAD coronary artery ligation. The animals were randomised to receive 1 ml basal medium (DMEM) without cells (group-I n = 5) or 3 x 10⁷ BMCs (group-II n = 10). BMCs was aspirated from donor rabbit and cultured in DMEM medium with 20% FBS. The adherent cells were propagated and labelled with lac-z, BrdU and DAPI prior to transplantation. The animals were administered 5 mg/kg cyclosporine 5 days prior to until 6 weeks after cell transplantation. The heart function was assessed by echocardiography. The animals were euthanised at 8 weeks after cell transplantation for histological studies.

Results: The preliminary data showed that lac-z and BrdU labelled BMC survived both in the normal and infarcted region. Interestingly, lac-z positive donor BMCs was observed in the walls of vasculature. Immunostaining with vonWillebrand factor VIII and smooth muscle actin showed that the BMCs transplanted region contained significantly higher blood vessels when compared with the DMEM injected group ($P < 0.001$). Preliminary heart function data revealed that there was improved left ventricular ejection fraction ($P = 0.008$) and fractional shortening ($P = 0.017$) after BMCs transplantation as compared to group-I DMEM injected animals.

Conclusion: Bone marrow derived stem cells survived in both normal and infarcted region of rabbit heart, formed blood vessels and may play a role in stimulating neovascularisation, with subsequently improved cardiac function.

I200/AH**NM23B, A Putative Tumour Metastasis Suppressor Gene, Is Down-regulated in HER-2/neu Positive Breast Cancer**SY NG¹, DH ZHANG², LK TAI¹, SK SETHI³, ESC SETHI²¹Department of Pathology & Laboratory, National University Hospital, Singapore, ²Department of Laboratory Medicine, National University Hospital, Singapore, ³Department of Laboratory Medicine, National University Hospital, Singapore

Aim: The HER-2/neu or c-erbB2 kinase receptor promotes cell proliferation, invasion and metastasis through the PI3K/AKT signal transduction pathway. Its overexpression in tumours is associated with poor prognosis. The aims of this work are to analyse the differential protein expression profiles between HER-2/neu-positive and -negative breast tumours and to identify gene transcriptions associated with HER-2/neu status.

Methods: We used cDNA microarrays to identify genes that are differentially regulated by HER-2/neu in breast tumours. Differential expression of such "discovered" genes were further confirmed and validated by real time RT-PCR, Western blot, reverse-phase protein arrays and tissue microarrays.

Results: A cohort of genes that were differentially regulated by HER-2/neu in breast tumours was identified. They include genes encoding mammaglobin 1, cytochrome c oxidase subunit VIa and ferritin which were significantly up-regulated, and those encoding NM23B (a non-metastatic cell 2 protein), olfactory marker protein, and small proline-rich protein 2B which were down-regulated in HER-2/neu positive breast tumour. Down-regulation of NM23B was further confirmed by Western blot, real time RT-PCR and reverse-phase protein array in 20 cases of frozen tissues. Immunohistochemical analysis of tissue microarrays constructed from 97 breast cancers showed the significant inverse relation of NM23B expression with HER-2/neu expression ($P = 0.032$).

Conclusion: cDNAs microarray analysis provides meaningful information of the transcriptomic differences characterizing these subsets. Down-regulation of NM23B, a putative tumour metastasis suppressor gene identified may be involved in cellular functions leading to the more aggressive phenotype in this subset of breast tumours.

I201/MP**Characteristics of Refusals in a Community-based Prevalence Survey of Neurological Diseases Among Older Singaporeans – Speeds Study**N VENKETASUBRAMANIAN¹, LCS TAN¹, S SAHADEVAN², JJ CHIN³, ES CHIN⁴, CY HONG⁵, SM SAW⁶¹Department of Neurology, National Neuroscience Institute, Singapore,²Department of Geriatric Medicine, Tan Tock Seng Hospital, Singapore,³Department of Geriatric Medicine, Tan Tock Seng Hospital, Singapore,⁴Research, National Neuroscience Institute, Singapore, ⁵Community,⁶Occupational and Family Medicine, National University of Singapore, Singapore, ⁶Community, Occupational and Family Medicine, National University of Singapore, Singapore

Aim: The SPEEDS study is a large community-based prevalence survey of Stroke, Parkinson's disease, Epilepsy and Dementia among adult Singaporeans aged 50 years and above. The overall participation rate was 67%. This paper reports the characteristics of those who refused to participate in this study

Methods: The study population comprised 24,978 subjects of Chinese, Malay and Indian descent, aged 50 years and above, living in the Ang Mo Kio, Bishan, Serangoon, Thomson, Toa Payoh and Yishun areas. They were randomly sampled, stratified by race in a Chinese:Malay:Indian ratio of 3:1:1. Trained interviewers sent an introductory letter followed by a phone call to potential subjects. The age, gender and race of those who refused to participate were recorded. Data were analysed using Epi-Info 6.

Results: Of the 24,978 possible subjects, 14,906 participated, 2699 were not eligible, 3370 remained uncontactable, and 3994 refused to participate. The refusal rate was 21.4% among eligible males and 14.6% among eligible females ($P < 0.001$). The refusal rate was highest among the Chinese (21.0%), lowest among the Indians (9.9%), with the Malays at 15.5%, ($P < 0.001$ by pair-wise comparisons). Refusal rates varied among the different age groups, highest (23.9%) among the 85 to 89 years age group and lowest (14.6%) among the 75 to 79 years age group.

Conclusion: Refusal rates are higher among males, Chinese and the very old. The reasons for this need further study. The lack of participation of subgroups of study populations could lead to a selection bias and consequent inaccurate findings. Funding: NHG, NMRC

I202/MP**Functional Magnetic Resonance Imaging of Working Memory in First-Episode Schizophrenia**HY TAN¹, WC CHOO², S GRAHAM³, C FONES¹, M FONES⁴¹Department of Psychological Medicine, National University of Singapore,²Cognitive Neuroscience Laboratory, Singhealth Research³Department of Psychology, National University⁴Singhealth Research Laboratories, Singapore

Aim: Working memory is adversely affected in schizophrenia. Existing imaging studies have characterized the functional anatomy of working memory in chronic schizophrenia where disease progression and therapeutic intervention might influence activation. Here, we evaluate cortical activation in response to two working memory tasks in first-episode schizophrenia to characterize the functional anatomy of early disease.

Methods: We studied 11 first-episode schizophrenia patients with less than 1 year duration of psychosis and who were treated with atypical antipsychotics. They were compared to 11 matched healthy controls. Each group performed two verbal working memory tasks while undergoing fMRI: one required maintenance of letters (L/TR); the other required manipulation in addition to maintenance (PLUS).

Results: Performance in both tasks was poorer but not slower in patients. Both patients and controls activated a predominantly left-sided frontal-parietal network, with PLUS eliciting activity of greater magnitude and spatial extent. With both tasks, patients showed less dorsolateral prefrontal cortex (DLPFC) activity, and greater ventrolateral prefrontal cortex (VLPFC) activity relative to controls. In patients but not in controls, performance was correlated with right VLPFC activation. Further, the task requiring manipulation resulted in greater VLPFC and less anterior cingulate activity in patients.

Conclusion: In patients with first-episode schizophrenia, tasks engaging verbal working memory reveal hypo- and hyperactivity in several prefrontal regions compared to controls. This suggests the presence of complex interactions between dysfunctional and adaptive responses within the prefrontal cortex, early in the course of schizophrenia.

I203/SC**The Use of NMP22 and Urine Cytology for the Surveillance of Patients With Superficial Bladder Cancer**WJ CHUA¹, E CHIONG¹, L LIEW¹, R KAMARAJ², K KAMARAJ¹¹Department of Urology, National University Hospital, Singapore,²Department of Surgery, National University of Singapore, Singapore

Aim: Superficial bladder cancer surveillance is expensive because of the intensive follow-up regime after the initial treatment. The aim of this study is to determine if nuclear matrix proteins (NMP22) can safely replace interval cystoscopy in detecting recurrence.

Methods: Thirty-four consecutive patients with biopsied proven low-grade (G1/G2) superficial bladder cancer (Ta/T1) were followed up for 2 years. Freshly-voided urine were collected for NMP22 and urine cytology test prior to cystoscopy at each follow-up visit.

Results: One hundred seventy-two urine samples were tested, 61 using NMP22 assay and 41 using NMP22 drop-test. 35 samples were tested with both and this showed 100% concordance. Bladder biopsies were taken from patients with suspicious lesion detected on cystoscopy or with positive cytology result. The results were compared with cystoscopy and histological findings. Using cut-off value of 8 U/mL, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of NMP22 in detecting TCC recurrences were 87.5%, 81.6%, 18.9% and 99.2% respectively. When combined with urine cytology, the sensitivity, specificity, PPV and NPV were 100%, 77.1%, 18.9% and 100%, respectively.

Conclusion: Our result shows that in surveillance of patients with low grade superficial bladder cancer, it is safe to replace interval cystoscopy with

NMP22 and urine cytology test. We recommend increasing the cystoscopy interval to 6 monthly for the 1st year and yearly from the 2nd year in this group of patients. This will translate to a 30% cost saving over 5 years.

I204/SC

Cortisol Metabolism in Polycystic Ovarian Syndrome

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Aim: In obesity in men, 11 β -Hydroxysteroid Dehydrogenase Type 1 (HSD1) activity in liver is impaired, contributing to enhanced metabolic clearance of cortisol and increased ACTH drive to adrenal androgen secretion. Preliminary evidence suggests similar disruption of hepatic 11 β -HSD1 in women with PCOS. In the ovary, granulosa cell 11 β -HSD1 activity and gene expression increases during follicular development. Failure of this increase in 11 β -HSD1 may contribute to hyperandrogenism and anovulation in PCOS. The aims of the study were to evaluate systemic and tissue-specific activity of 11 β -HSD1 (converts inactive cortisone to active cortisol) in PCOS.

Methods: In 10 control and 11 PCOS patients undergoing IVF, we measured: 1) Systemic excretion of glucocorticoid and androgen metabolites by GCMS. 2) Hepatic 11 β -HSD1 activity after dexamethasone suppression followed by oral administration of cortisone and serial venepuncture for plasma sampling over 180 mins. 3) Cortisol metabolism by ovarian granulosa cells obtained during IVF. 4) Follicular fluid concentration of glucocorticoids.

Results: Systemic 11 β -HSD1: Urinary excretion of glucocorticoid metabolites were not different between the 2 groups. Hepatic 11 β -HSD1: After dexamethasone, plasma cortisol was equally suppressed in both groups. Conversion of oral cortisone to cortisol was significantly impaired in PCOS. Ovarian 11 β -HSDs: 11 β -reductase activity was significantly lower in PCOS compared to controls (mean $\pm$ SE % conversion cortisone to cortisol: 29.7 $\pm$ 2.3 vs 22.5 $\pm$ 2.3, $P < 0.005$).

Conclusion: The data demonstrates a tissue-specific (liver and ovary) impairment of 11 β -HSD1 activity in-vitro. Altered 11 β -HSD1 activity in PCOS may be amenable to novel therapeutic manipulation.

I205/SL

Resistance to Fas-mediated Apoptosis in Gestational Trophoblastic Disease: Implications for Immune-privilege

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Aim: An immune-privileged site occurs when allogenic tissue grafts have the greater propensity for prolonged survival in the host tissue. In this context, the survival and proliferation of malignant trophoblasts in the gravid uterus is presently unclear. Current evidence suggests that ligation of the cell surface molecule Fas and its natural ligand, FasL could lead to induction of apoptosis in several cell types. In this study we investigated the co-expression of Fas/

FasL in choriocarcinoma (CC) cell lines.

Methods: Using the well characterised BeWo and NJG trophoblast cell lines we provide evidence for immune-privilege using flowcytometry, MTT assays, co-culture and RT-PCR analyses.

Results: The mean fluorescence intensity of Fas receptors for NJG and BeWo were 1.47/0.5 and 1.59/0.4 (mean/SD) while that for Fas positive Jurkat cells was 25.6/3.1 suggesting a down regulated Fas expression in CC. In BeWo and NJG cells the mean cell viability declined to about 58% and 63% of the control in the presence of anti-Fas mAB while Fas sensitive Jurkat cells showed viability of only 10% using MTT assays. This resistance to Fas-mediated apoptosis in CC cells is reversed in the presence of cycloheximide which further decreased viability to 36% and 32% respectively ($P < 0.05$). In co-culture experiments these CC cells induced apoptosis in Fas-sensitive Jurkat cells, thereby imparting immune-privilege.

Conclusion: Our data indicate that CC cells evade immune attack by down regulation of the Fas receptor and by killing lymphocytes through expression of FasL. The potential involvement of Fas/FasL in malignant transformation is of significance in evasion of cell death and promotion of proliferation and metastasis of such tumours.

I206/SL

Hypoxia Downregulated VCAM-1 Expression and Secretion in Human Placental Trophoblasts

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Aim: Many processes that are involved in cellular invasion, including blastocyst implantation, placental development and rapidly growing tumours occur in reduced oxygen environments. It has been surmised that oxygen tension could regulate the cytotrophoblast ability to differentiate, and as a consequence, to express proteins that are critical for placentation. The objective of this investigation was therefore to test the hypothesis that placental tissues and trophoblast cells in culture, under low oxygen tension, release angiogenic factors which could affect vascular behavior and invasive potential, thus providing a link between abnormal placentation and maternal vascular abnormality. In this investigation, we examined the expression and secretion of a pro-angiogenic molecule VCAM-1, a member of the immunoglobulin superfamily with or without hypoxia in placenta

Methods: Functionally active term placental explant culture and trophoblast cultures were used to demonstrate the secretion profile of VCAM-1 and the real-time quantitative RT-PCR technique was employed to demonstrate the mRNA expression under both normoxic and hypoxic conditions

Results: A significant decrease in the secretion ($P < 0.05$) and mRNA expression ($P < 0.03$) of VCAM-1 from both term placental explants and trophoblast cultures subjected to hypoxia in vitro was observed

Conclusion: Since the primary defect in uteroplacental insufficiency is placental maldevelopment probably associated with hypoxia in situ, this study provides molecular evidence to indicate that the decreased expression and secretion of VCAM-1 could have an important role in pathological conditions such as pre-eclampsia and IUGR

Poster Presentation Abstracts – Day Two

I/II – Day One/Day Two; xxx – Poster Board Number; AH – Allied Health/Health Sciences/Paramedical Disciplines; CPIP – Clinical Practice Improvement Programme; MPC – Medicine/Paediatrics (Clinical based); MPL – Medicine/Paediatrics (Laboratory based); NUR – Nursing; SC – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Clinical based); SL – Surgery/Obstetrics & Gynaecology/Dentistry/Ophthalmology (Laboratory based)
Layout of Posters: See Programme Guidebook pages 21-28

II001/AH

Mobile Crisis Service: A Pilot Project of the Institute of Mental Health

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Aim: Recent trends in mental healthcare worldwide show an emergence of community crisis intervention services. Such services are seen to support the development of alternatives to hospitalisation in the provision of acute psychiatric care. In Singapore, the Institute of Mental Health has recently launched a Mobile Crisis Service to augment the existing community services. Broadly, the service aims to reduce the impact of mental health emergencies through immediate response to crises at the community level.

Methods: There are 2 components to the service: a crisis hotline offering an available source of support for mental patients in crisis immediately, and a mobile crisis team (MCT) that does on-site crisis interventions aiming to de-escalate situational crises in order to prevent unnecessary visits to the hospital's emergency department.

Results: From January to May 2004, there was a total of 856 phonecalls to the hotline, with 86 home visits by the MCT. About half of the calls were for phone counselling. The majority of cases attended to by the MCT were for relapsed and/or violent patients. 27 cases were resolved on-site while 41 needed admission. Of these, 8 were arrested and brought in by the police.

Conclusion: Timely intervention by professionals during mental health emergencies can help prevent the escalation of crises to disasters. Also, by offering a ready source of support and practical assistance, it is hoped that caregiving burden can be alleviated.

II002/AH

Neural Cell Adhesion Molecule Mediated Neurite Outgrowth in Relation with Members of Protein Kinase C

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Aim: It is well known that the neural cell adhesion molecule (NCAM) is involved in cell migration, neuronal differentiation, neurite outgrowth and synaptic plasticity. However, the precise effect of various members of NCAM on neurite outgrowth is still not clearly defined. This study is aimed to find out the relation of NCAM 140 and NCAM 180 in TPA induced morphological changes in neuroblastoma cell lines.

Methods: We have got 3 different cell lines, where the NCAM 140 and NCAM 180 levels of protein expression are different. These cell lines were treated with TPA for 2 to 4 days, and cell morphology and different protein expressions were studied. Neuronal differentiation was assessed by microscopic observation and protein expression which were determined by western blotting, immunoprecipitation and immunohistochemical staining.

Results: Neurite outgrowth in SH-SY5Y (human neuroblastoma cell line) cells treated with 16nM TPA showed longer, hard processes with growth cone, whereas T18 cells (where NCAM 180 is mainly expressed) had soft neurites with lots of small branching. Transfection of NCAM 140 to T18 cells convert neurite behaviour into SH-SY5Y cell like with harder and longer growth cone morphology. We also found out co-localisation of NCAM140 with PKC members in different cell lines by immunoprecipitation, immunohistochemistry and western blot techniques.

Conclusion: Our findings suggest that neural cell adhesion molecules associate with certain members of protein kinase C prominently on growth cone formation.

II003/AH

Use of Antilipemic Drugs in National Healthcare Group (NHG) Singapore

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Aim: To assess the use and cost of antilipemics in NHG.

Methods: Dispensing data for antilipemics for AH, NHGP, NUH and TTSH from January to September 2003 was extracted and analysed using WHO ATC/DDD methodology (version 2004).

Results: Statins accounted for 90% (88%-92%) whereas fibrates accounted for 10% (8%-12%) of total DDDs. Lovastatin and simvastatin predominated the antilipemics use (83% of total DDDs). Although the usage of atorvastatin and pravastatin was less than 7% of total DDDs, they accounted for almost 50% of the total cost for antilipemics. The total usage and cost of antilipemics were highest in NHGP. Atorvastatin and pravastatin were used more commonly in NUH and TTSH (22% and 17% respectively) and resulted in higher (2-3 times) average daily cost as compared to AH & NHGP (2% for both institutions). The analysis also revealed that NUH and NHGP have a higher average daily cost of bile acid sequestrants and nicotinic acid derivatives respectively, due to the use of more expensive agents in these groups of drugs.

Conclusion: Generally the utilisation profile of antilipemics was similar across the 4 institutions. Statins were the leading antilipemics followed by fibrates. Some institutions used more expensive antilipemics compared to the others. This has significant cost implication to patients as well as institutions, and warrants closer analysis of patterns of antilipemics' usage to identify the reasons and outcomes for the use of these agents and to identify measures that may be taken to rationalise the prescription of these agents.

II004/AH

Mapping Census of Population 2000 (Singapore) for Health Planning

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Aim: Healthcare utilisation is very much driven by demographics of the population of an area or region served. The Singapore Census is an essential resource for understanding the characteristics of the population and households. The NHG has developed an environment that combines the Census data and the geographic information system to aid its provision of healthcare services.

Methods: Data from the Census of Population 2000 by Ministry of Trade & Industry, Singapore was transformed spatially using the GIS. The distribution of demographic parameters (age, gender, ethnic group, housing type and income) was displayed geographically using the 55 DGP zones.

Results: Distribution of population: 58% of the total population was from 10 DGP zones; top 5 zones being Bedok, Tampines, Jurong West, Hougang and Woodlands. Age: Overall, the elderly population (65+ years) comprise 7.3% of the resident population. Highest proportion of elderly patients is from Outram, Kallang and Bukit Merah. Housing type: Overall, 85% of dwelling units were HDB apartments, private apartments being 6% and landed properties 7.5%. Smaller HDB flats (1-3 rooms) were more concentrated in Outram, Queenstown and Bukit Merah. Income: For resident working persons (15+ years), 27% earned less than \$1500/mth. The areas with highest percentage earning <\$1500/mth were Outram, Kallang, Rochor, Bukit Merah and the Downtown Core.

Conclusion: The spatial display of such demographic information geographically allows visualisation of areas of differing healthcare needs and is useful for national planning of appropriate allocation of resources and siting of new services where a gap is identified.

II005/AH

Biomedical Technology in Progress with Responsibility: An Ethical Legal Social Review of Human Stem Cell Research, Therapeutic and Reproductive Cloning in Singapore

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Aim: The cloning of human embryos for therapeutic research carries controversial ethical issues. This paper discusses human stem cell research, with a focus on clinical research compensation related injuries. An analytical and critical discussion is also undertaken on the earlier "Regulation of Biomedical Research Bill" and the current "The Human Cloning and other

Prohibited Practices Act 2004”.

Methods: We reviewed the major literature on our subject and carried out a comparative study of the major countries' viewpoints, as well as discussed the recommendations by the Singapore Bioethics Advisory Committee (BAC), interjected with our arguments, current opinions and suggestions.

Results: Our study revealed diverse opinions on the ethical acceptability of human stem cell research among various countries. In Singapore, the BAC examines and makes recommendations on scientific, ethical, legal and social issues arising from biomedical research and development. The BAC's fundamental approach is to balance 2 ethical commitments – to advance human life by curing diseases and to protect human life. To formulate its recommendations, it relies on the twin guiding principles that the results of biotechnology must be 'just' and 'sustainable'.

Conclusion: Human stem cell research should be regulated and monitored in accordance with the relevant ethical considerations. There should be an international convention on bioethics and human stem cell research so that a consensus can be reached internationally with minimum standards and safeguards to be placed at national levels.

II006/AH

Singapore Children Emotional Distress Scale: Examination of Psychometric Properties and Identification of Clinical Cut-offs
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Aim: Recognising the lack of a brief parent-rated tool for screening children's emotional distress locally, Parker and colleagues (2001) developed a Singapore Children Emotional Distress Scale (SCEDS). Its psychometric properties (factor structure, construct validity, discriminant functions), function as a brief measure, and appropriate cut-offs were examined with a clinical sample in the present study.

Methods: Participants were 143 parents whose children were attending the Child Guidance Clinic. Of these children, 21 had stress-emotional disorders, 55 had disruptive behaviours, 40 were normal variants, and 27 had other diagnoses. The SCEDS and the Child Behavioural Checklist (CBCL, Achenbach & Edelbrock, 1983) were administered.

Results: A 2-factor structure was found for the SCEDS. Its internalising factor was found to correlate higher with the CBCL internalising factor compared to its correlation with the CBCL externalising factor, while its externalising factor correlated higher with the CBCL externalising factor compared to its correlation with the CBCL internalising factor, supporting its construct validity. Both the SCEDS internalising and externalising factors were found to discriminate between children with stress-emotional disorders, disruptive behaviours and children who were normal variants appropriately, supporting its discriminant functions. Compared to the CBCL internalising factor, the SCEDS internalising factor was found to be a stronger predictor of clinicians' diagnoses of stress-emotional disorders. Suggested cut-offs for the SCEDS internalising and externalising factors were 3 and 1 respectively.

Conclusion: The SCEDS internalising factor might function as a potential screening tool for children's emotional problems in Singapore.

II007/AH

The Impact of Neurocognition and Premorbid Adjustment on Psychosis in Singapore

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Aim: This study aims to examine the relationships between premorbid functioning and neurocognitive deficits in first-episode psychosis.

Methods: Patients with first-episode psychosis were assessed with validated

clinical instruments, including a neurocognitive battery and measure of premorbid functioning. The Wisconsin Card Sort Test (WCST) and the Trail-Making Test (TMT) assessed executive functioning. The Continuous Performance Test (CPT-II) and Digit Span (WAIS-III) measured attention. The National Adult Reading Test (NART) and Ravens Progressive Matrices (RPM) were used to assess IQ functioning.

Results: Thirty Singaporean patients (mean age of 24.4 years, SD = 5.7) of whom 21 (61.8%) were males participated in the study. The average duration of untreated psychosis (DUP) reported was 21.7 months (SD = 32.3), median DUP was 11 months. Statistically significant associations were found between premorbid functioning scores and scores of CPT ($r = 0.427$, $P = 0.03$), TMT ($r = -0.458$, $P = 0.016$) and WCST ($r = -0.444$, $P = 0.029$). Multiple regression analyses further supported the findings.

Conclusion: Results support the neurodevelopmental hypothesis of schizophrenia, postulating that early insult to the brain may lead to neurocognitive deficits and poor premorbid functioning.

II008/AH

Effectiveness of Very Low Calorie Diet (VLCD) in Weight Reduction for Super Obese Patient Prior to Laparoscopic Gastric Banding

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Aim: 1) To reduce the weight of a super obese in-patient with BMI 90 prior to Laparoscopic Gastric Banding thereby reducing mortality risk; 2) to improve co-morbidities i.e. OSA with Type 2 respiratory failure and osteoarthritis; 3) to improve overall quality of life.

Methods: The patient was prescribed VLCD (*Optifast) for 12 weeks prior to laparoscopic gastric banding. The risks involved with taking VLCD were informed and consent was taken. Laboratory investigations included blood glucose, renal panel, uric acid, liver function tests, ECG, urine ketones and blood gases. Diet: Patient was educated on the use of VLCD. 3 packets of Optifast were given daily in accordance to meal times (providing 456 kcal, 51.9 g protein, 6.9 g fats). Lunch and dinner included 100 g vegetables with no dressings or sauces. Patient was encouraged to take 2 to 3 L of water daily. No other food was allowed. Progress: Patient was photographed weekly over the 11 weeks and weighed daily to monitor the rate of weight reduction. Relevant laboratory investigations were repeated according to protocol.

Results: After 11 weeks of VLCD treatment, the patient lost 50.3 kg. Improvement in blood gases and patient mobility were noted. Oxygen could be weaned off.

Conclusion: The Very Low Calorie Diet was an effective method of weight reduction for this super obese patient. Motivation and close monitoring by doctors and dieticians were mandatory during treatment. Patient's co-morbidities and overall quality of life improved after having lost 50.3 kg.

II009/AH

Nutritionally-variant *Escherichia coli* from Blood Culture

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Aim: *Escherichia coli* (*E. coli*) belong to the family Enterobacteriaceae, members of which are biochemically active and grow readily on ordinary media. In this hospital 11.2% of total isolates from blood culture between January and April 2004 were *E. coli*. An isolate of Gram-negative bacilli, which grew reluctantly on ordinary media, was obtained from the anaerobic blood culture of a septic 70-year-old patient. After overnight incubation at 35°C supplemented with 5% CO₂, this isolate produced pinpoint β-haemolytic colonies, with enhanced growth in anaerobic atmosphere. Direct Gram stain from the blood culture bottle and of the colonies showed Gram-negative bacilli. There was no growth on direct antimicrobial susceptibility tests done on Mueller-Hinton (MH) Agar and the direct conventional biochemical identification tests were non-reactive except for lysine decarboxylation test, which was positive. Oxidase test was negative. No conclusive identification was obtained using various commercial identification kits. Initial suspicion was that this isolate is fastidious in its requirements for growth and that it

belongs to the HACEK group of organisms.

Methods: This isolate was inoculated in nutrient broth and incubated for 4 hours at 35°C supplemented with 5% CO₂ to initiate its growth. A fresh set of biochemical identification and antimicrobial susceptibility tests was done using the broth in which growth had been initiated in a capnophilic atmosphere.

Results: The biochemical identification tests yielded a conclusive identification. Growth on MH Agar was satisfactory and antimicrobial susceptibility test results interpretable.

Conclusion: The isolate was identified as a microaerophilic, extended spectrum β -lactamase producing *E. coli*.

II010/AH

Incidence of Acute Transfusion Reactions, Untoward and “Near Miss” Events During Transfusion in an Acute Care Hospital

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Aim: A retrospective study was done to classify all acute transfusion reactions, untoward and “near miss” events during transfusion in an acute care hospital.

Methods: Acute transfusion reactions, untoward and “near miss” events reported between year 2001 and 2003 were consolidated. Acute transfusion reactions were categorised based on the standards of the American Association of Blood Banks criteria.

Results: 229 acute transfusion reactions were reported for 67 819 units of blood and blood components transfused. 50% (115/229) of the reactions were allergic reactions, 30% (69/229) were Febrile Non-Haemolytic Transfusion Reactions (FNHTR), 18% (41/229) were non-specific reactions which cannot be classified as FNHTR as patients had a rise in temperature of less than 1°C, and 2% (4/229) were anaphylactic reaction. Twenty-seven “near miss” and 2 untoward events (blood administration to wrong patient) were reported. Of the 27 “near-miss” events, 67% (18/27) of the events were due to errors during specimen collections, 11% (3/27) were due to incorrect blood component issued owing to incorrect product collection form from the ward and 4% (1/27) were due to incorrect methods of blood product transportation. Blood bank errors including clerical error in results entry and transcription, and issuances of incorrect blood components were responsible for 11% (3/27) and 7% (2/27), respectively.

Conclusion: There was no acute haemolytic transfusion reaction, transfusion associated sepsis or transfusion related acute lung injury reported. Near misses occurring during transfusion process continued to be a significant problem. These were mainly due to human errors. Strict adherence to the Standard Operating Procedure is mandatory to avoid these errors.

II011/AH

Cytospin Evaluation in Urine Cytology: Are Two Slides Better than One?

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Aim: Preparation of duplicate slides for urine cytology analysis is a common practice. In this study, we assess the feasibility of using a more economic single cytospin preparation.

Methods: Duplicate cytospin preparations of 120 urine samples received over a 6-month period in 2003 were reviewed. The 240 slides were randomised and assessed blind by 2 independent observers. Parameters evaluated included smear quality (cellularity/cell distribution), satisfactory rate, sensitivity and specificity. For each sample pair, the less informative of the 2 samples was used for comparison. Histology reports for 26 cases were available.

Results: Thirty-one cases showed discrepancies in diagnosis: 14 unsatisfactory single slides yielded diagnostic material in the respective duplicate slides, 16 negative single samples revealed malignant or atypical diagnoses in the second slide and 1 atypical case showed malignant cells in the second sample. The sensitivity, positive predictive values (PPV) and negative predictive values (NPV) of dual cytospin preparations were 94.7%, 94.7% and 83.3% respectively, superior to the corresponding single slide preparation (66.7%,

92.3% and 45.5% respectively). The specificity was similar with both methods (83.3%).

Conclusion: Using dual cytospin slide preparations significantly improves specimen adequacy, sensitivity, PPV and NPV of urine cytology samples. These advantages more than offset the slightly reduced cost saved in single slide preparation.

II012/AH

Different Approaches to Improve Smoking Cessation Attempts Among Smokers

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Aim: Smoking continues to be a preventable cause of death with smokers deluded to the harmful effects of tobacco, due to the physical and psychological pleasures derived. The process of quitting begins when the precontemplating smoker (not interested to quit) becomes a contemplator of quitting. The low quit rate in Singapore is chiefly due to smokers seeking advice only when they become contemplators. Therefore, our team seeks to improve smoking cessation attempts by encouraging more precontemplators to quit. This is done through implementing group counselling and piloting inpatient smoking cessation counselling (in 3 wards), in addition to our existing outpatient counselling work.

Methods: Group counselling was conducted in institutions that recruited our services, whilst patients noted to be smokers on admission to AH were given inpatient counselling. Smokers who refused counselling were excluded from data collection. Smoking cessation counselling involved obtaining the smoking history, assessing the level of addiction, providing advice and medication (where appropriate). Smokers were followed up for at least 2 months.

Results: Seventy-seven smokers were counselled over 12 months (46 group and inpatient, 31 outpatient cases). The number of smokers counselled was 1.5 times greater than the previous year (28 outpatient cases). The number of smokers who attempted to stop smoking was 4 times greater, with 29 cases (38%) from outpatient, group and inpatient counselling compared with 6 cases (7.8%) from outpatient counselling alone (based on the previous year).

Conclusion: Group and inpatient smoking cessation counselling are effective approaches to improve smoking cessation attempts.

II013/AH

Reducing Medication Administration Documentation Errors and Discharge Prescriptions Transcribing Errors in the In-patient Setting – A Multi-disciplinary Approach

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Aim: Nurses administer medications to in-patients and document the activity by signing the relevant columns of the in-patient medication record (IMR). Doctors ink out discharge prescriptions based on medications ordered in the IMR. Medication administration documentation errors by nurses can lead to miscommunication and inappropriate medication regimen being delivered. Transcription errors from the IMR to discharge prescriptions can likewise lead to wrong medication regimen being dispensed to patients upon discharge. The aims of the study are to reduce medication administration documentation errors and discharge prescription transcribing errors to improve patient safety.

Methods: A new IMR was designed based on a survey and suggestions of all staff (medical, nursing and pharmacy) involved in the use of the IMR. The new IMR was put on trial in 3 wards of mixed disciplines and gender.

Results: Regular, daily audits of the IMR and data from pharmacist-physician interventions over ambiguous discharge prescriptions that did not match with the IMR showed a mean of 4.2 documentation errors and 3.2 ambiguous discharge prescriptions per ward monthly. During the trial, staff was briefed on the use of the IMR and audit data collected one week after commencement. Both kinds of errors were reduced to nil in the trial wards.

Conclusion: A multi-disciplinary involvement in the revamp of the IMR is associated with a reduction in the 2 kinds of medication errors. Further audits and review of processes are required to verify its potential.

II014/AH**Physiotherapy Management of Kartegener's Syndrome—A Case Report**CLW NG¹, MTL YEUNG¹, CM LOO²¹Department of Physiotherapy, Singapore General Hospital, Singapore,²Department of Respiratory Medicine, Singapore General Hospital, Singapore

Aim: Kartegener's syndrome is an autosomal recessive hereditary condition, of unknown aetiology, comprising of a triad of transposition of the viscera, abnormal frontal sinuses producing sinusitis, bronchiectasis and immotility of the cilia leading to dyspnoea, productive cough and recurrent pneumonia. The usual aim of physiotherapy was to improve mucociliary clearance.

Methods: We present a patient with Kartegener's syndrome, with heart on the right side. He had repeated chest infections with increasing dyspnoea and exercise intolerance. The patient, a 57-year-old man (body mass index 18.6) on long-term oxygen therapy (2 L/min), was admitted to the hospital for assessment of rehabilitation potential. On admission, peak oxygen consumption (VO₂) was 751 mL/min (41% predicted) on symptom-limited ergometry testing. Six-minute walk distance (6MWD) was 138 m. Chronic Respiratory Questionnaire (CRQ) and Medical Outcomes Trust Short-Form (SF)-36 were administered.

Results: The patient underwent aerobic exercise training (cycling and treadmill walking), extremities strengthening and general thoracic wall flexibility exercises. He completed 18 sessions in the 2 weeks' hospitalisation. His peak VO₂ was 831 mL/min (46% predicted; +10% improvement) and 6MWD 160 m (+16%). His CRQ score improved from 55 to 73 (+33%) and SF-36 from 119 to 205 (+72%). Improvement in CRQ was recorded in all 4 dimensions. Improvement in SF-36 was primarily in physical functioning, general health, vitality and mental health domains.

Conclusion: In conclusion, this case report highlights the role of physiotherapy in improving exercise tolerance in a patient with Kartegener's syndrome, rather than focussing on mucociliary clearance.

II015/AH**Quality Correlates with 6-Minute Walk Distance in Patients Undergoing Pulmonary Rehabilitation**

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Aim: The aim of this study was to correlate aspects of quality of life with functional performance in patients undergoing pulmonary rehabilitation.

Methods: Fifty-five patients of mean age 63.2 years (SD 11.5) with chronic respiratory diseases (chronic obstructive pulmonary disease, 56%; bronchiectasis, 9%; asthma, 9%; primary emphysema, 6%; others, 20%) participated in 18 sessions of pulmonary rehabilitation over 6 weeks. The programme included aerobic exercise training and upper/lower extremity strengthening. Chronic Respiratory Questionnaire (CRQ) and 6-minute walk distance (6MWD) testing were administered before and after the 6-week rehabilitation programme.

Results: Both CRQ total scores and 6MWD were significantly correlated (start of the programme, $r = 0.40$, $P = 0.004$; end of the programme, $r = 0.62$, $P < 0.001$).

Conclusion: Both dyspnoea scores predicted almost 31% of the variance in 6MWD. With post-rehabilitation dyspnoea score accounting improvement in functional performance seeming to correlate with overall improvement in quality of life, dyspnoea appears to be the most important predictor of functional performance.

II016/AH**The Assertive Community Treatment Programme in the Institute of Mental Health, Singapore**

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Aim: The Assertive Community Treatment (ACT) Programme, launched on 1 Nov 03, provides community-based psychosocial rehabilitation for people with disabling severe mental illness, with the aim of improving their social

and occupational functioning. Through this poster, we hope to increase the awareness about this programme, and to report the demographic profile of our patients.

Methods: Forty-one patients, with severe mental illness and a significant period of hospitalisation in the preceding year, were selected from 52 referrals received from IMH psychiatrists over a 7-month period from 1 Nov 03 to 30 May 04. For each patient, the ACT team (comprising a psychiatrist, medical officer, medical social work, occupational therapist and 4 community psychiatric nurses) has planned an individualised psychosocial rehabilitation programme, which covers areas such as training in activities of daily living and psycho-education. We report the profile of our patients below.

Results: Most patients are in the age group 40-50 (34.1%) with a mean age of 42. Majority of them (87.8%) are Chinese. The number of male and female patients are roughly equal. Except for 2 patients with schizoaffective disorder and bipolar disorder, the other patients have schizophrenia. The average number of hospitalisation-days in the preceding year is 85 (range, 12-365), and their duration of illness ranges from 1 to 43 years.

Conclusion: The ACT Programme is the first local home-based psychosocial rehabilitation programme. The patients' outcome will be analysed after the first year, including the rates of recidivism and re-hospitalisation-days compared to the previous year.

II017/AH**Validity of 'Standard' Dose-prescription Parameters for Palliative Spinal-irradiation – An Evaluation on Asian Patients**KM LEE¹, JF FU², SK SOLLIN², LW KHIN³¹Department of Radiation Oncology, National University Hospital, Singapore,²Radiation Oncology, Tan Tock Seng Hospital, Singapore,³Acupuncture Clinic, Ministry of Health, Singapore, Singapore

Aim: Spinal-metastasis presents often with debilitating backache and even neurological complications. As treatment usually involves palliative spinal-irradiation, 'standard' radiotherapy-prescription parameters should be validated for application on Asian patients.

Methods: Fifty radiotherapy-prescriptions for 46 patients treated at a single institution were reviewed with assessment of irradiated spinal-volumes and construction of dose-volume histograms (DVH) using CT-planning. Spinal depths from posterior skin to posterior spinal-canal (Point A), anterior spinal-canal (Point B) and anterior vertebral border (Point C) were recorded at 8 levels: thoracic (T2,5,8,11), lumbar (L1,3,5) and sacral (S2).

Results: A direct posterior-anterior (PA) portal with 6 MV photons was used for all but one treatment (10 MV). Average dose, fractions and reference depth were 19.7 Gy (8-30 Gy, SD 5.7), 5.5 (1-10 fractions, SD 2.5) and 5.1 cm (4.6-7 cm, SD 0.4) respectively. There was marked variation in spinal-depth at different levels. The average and maximum for point C were 8.7 cm (L1) and 10 cm (L5) respectively, 0.9 to 1.9 cm less than findings in a Canadian-based study. There was no difference in the average spinal-depths between males and females. However, lumbar-spinal depth at point C is deeper by 0.6 to 0.9 cm in males at L1 ($P = 0.01$), L3 ($P = 0.03$) and L5 ($P = 0.06$), contradicting findings of the Canadian-based study. The mean-volume of irradiated-spine was 442 cc encompassing 6 vertebrae on average. Volume per vertebra-body (VVB) for T-spine was 49 cc in contrast with 110 cc for L-spine ($P < 0.001$). Despite this, radiation dose-range of 68.5% to 117.7% within irradiated spine remained consistent between levels averaging 97.7% of the prescribed dose.

Conclusion: Three-dimensional CT-planning is useful in correlating spinal-anatomy with radiation dose-distribution for validation and optimisation of radiotherapy techniques.

II018/AH**Investigating Gender Differences in Following Severe Traumatic Brain Injury**T TAN¹, J LIM², KK LEE³, HB WONG², TT YEO⁴, I NG⁴¹Department of Research, National Neuroscience Institute, Singapore,²Clinical Trial Epidemiology Research Unit, Singapore, ³Department ofNursing, National Neuroscience Institute, Singapore, ⁴Department of Neurosurgery, National Neuroscience Institute, Singapore

Aim: The objective of this study was to investigate if there are possible gender

differences in relation to outcome following closed severe Traumatic Brain Injury (TBI) in a predominantly Asian population.

Methods: A retrospective study was conducted using our prospectively maintained severe TBI database. Four hundred and eighty patients with severe head injury admitted into our neurosurgical intensive care were studied. All patients were managed according to the "Guidelines to the management of severe traumatic brain injury". A dichotomised Glasgow Outcome Score was used to measure the outcome of patients 6 months post injury.

Results: There were 100 females and 380 males. The median age of female patients was significantly higher than male patients. There was a slight but not significantly higher overall case fatality rate and proportion of patients who made a poor recovery (at 6 months post injury) in females. Crude and adjusted odds ratios revealed that only increasing age, presence of pupil abnormality and a lower GCS score were significantly predictive of death and poor outcome.

Conclusion: Both odd ratios indicate that mortality and poor outcome did not differ significantly between men and women.

II019/AH

PKC- β Expression is Highly Associated with Patient Survival in Diffuse Large B-Cell Lymphoma and Glioblastoma: Use of Public Microarray Datasets and its Implication in Clinical Drug Development for Enzastaurin HCL[®]

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Aim: PKC- β is an isoform of protein kinase C, a family of serine-threonine kinases involved in a wide range of signal transduction pathways such as cell proliferation, cell differentiation and apoptosis. PKC- β has been shown to be one of the most prominently over-expressed genes in fatal/refractory DLBCL patients. Its role in tumour development and angiogenesis makes it a potential therapeutic target in cancer. Enzastaurin HCL is a potent and selective inhibitor of PKC- β . The compound exhibited antiangiogenic activity in a preclinical animal model and is well tolerated in toxicology studies.

Methods: In this study we analysed public gene expression profiling data on different types of cancer to investigate if PKC- β gene expression is correlated with patient survival.

Results: Our analysis has demonstrated that high PKC- β expression has a strong correlation with poor patient outcome in DLBCL, confirming the observations published in previous publications on these datasets. We have also linked PKC- β expression with clinical prognostic markers like the International Prognostic Index for DLBCL. A similar demonstration of a correlation between PKC- β expression and poor survival was observed in glioblastomas. Similar analysis of MCL and other solid tumours like non-small cell lung cancer did not yield similar associations between PKC- β and survival.

Conclusion: The analysis of public gene expression data and its correlation with patient survival suggests that inhibiting PKC- β in patients with DLBCL and glioblastomas may provide a clinical benefit.

II020/CPIP

Improvement of Turnaround Time for Mammography

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Aim: In November 2002, we aimed to reduce the turnaround time (TAT) for a diagnostic mammographic examination to within 1 hour for at least 90% of patients by 6 months. A preliminary survey showed that 67.1% of patients spent more than an hour within our department. We wanted to minimise waiting time for these highly anxious women.

Methods: The team used CPIP methodology. A flowchart for mammography, a cause-and-effect analysis of the long waiting time and a pareto chart were constructed. Main problems included wrong arrival time of patient (26%), long billing time (25%), many interruptions during the procedure (16%), film viewing area too far from mammography room (12%) and many extra views

(11%). Interventions included reminders to patients to come on time, streamline billing and payment, relocation of film viewing area, closer supervision and CME activities for junior staff and diversion of phone enquires. We went through several PDSA cycles.

Results: The project was interrupted in March 2003 due to the SARS outbreak. By June 2003, the target of 90% was achieved. Periodic surveys showed an improvement maintained at above 90%. A run chart of the results is displayed in the mammography and film viewing rooms to remind, encourage and motivate the staff. The mean TAT was reduced from 61.9 minutes to 51 minutes. This savings of 10.9 minutes per patient translates into an additional 2 cases per day and additional revenue for the hospital.

Conclusion: Improvement of services for the benefit of our patients is possible with the active participation of stakeholders.

II021/CPIP

Reduction of Peripheral IV Phlebitis in a Surgical Unit

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Aim: The use of peripheral intravenous device (PIVD) is an integral part of patient care. Insertion of a PIVD predisposes the patient to numerous local and systemic hazards, of which phlebitis is one. In May 2002, the point prevalence phlebitis rate of a surgical unit (comprising 3 wards) in TTSH was 26.3%. A CPIP was undertaken in December 2002 to reduce the phlebitis rate by 50% in 3 months.

Methods: A multidisciplinary team was formed to reduce the phlebitis rate. A literature review was done to explore the causes of phlebitis. This was followed by team brainstorming, healthcare workers multi-voting and an audit to identify the leading causes of phlebitis in the unit under study. Interventions targetted the frequent causes identified, namely the speed of administration of medication, flushing of PIVD after medication administration, IV bolus against recommendations and incorrect dilution of drugs. An antibiotics information chart and a list of drugs not for bolus administration were compiled and all categories of staff were educated on the findings of the audit, as well as the above recommendations.

Results: The point prevalence phlebitis rates were 14.6% in January 2003 (a reduction by nearly 50%) and nil in January 2004.

Conclusion: Staff awareness is important in the reduction of phlebitis. Resources on correct drug preparation and administration should be made readily available for reference. It is also important to conduct regular in-service education to reinforce recommendations and correct practices.

II022/CPIP

To Reduce the Inpatient Fall Rate

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Aim: The inpatients' fall rate was increasing in the year 2002. Thus, a clinical project improvement team was formed in October 2002 to reduce the fall rate.

Methods: The team analysed and collated data. The root causes were patients climbing out of bed, losing balance and falling due to their medical condition. The team utilised 3 Plan, Do Study, Act (PDSA) cycles and piloted various solutions before hospital-wide implementation. The Fall Risk Assessment, Fall Prevention Action Plan and Fall Prevention Protocol were revised. Patients who had undergone surgery, invasive procedure or treatment, had been transferred from another department or had a significant change in condition were reassessed for the risk profile. Green wrist tags and highlighted "FALL PREVENTION" tags placed on the information panel were introduced for easy identification of high-risk patients. Bed pan and urinal rounds at periods of high fall rate of noon, 2200 hours, 0200 hours and 0600 hours and hourly nursing rounds were introduced. Carers or relatives were encouraged to accompany the patients. Nurses were instructed to ensure that all fall prevention interventions were observed before taking over care of patients during shift change. Three road shows and 8 fall prevention workshops were conducted to increase awareness and compliance among nurses. A fall prevention committee was formed and auditors were nominated to conduct monthly cross ward audit for compliance. The strategy for spreading includes

training for new staff.

Results: The fall rate decreased by 15%.

Conclusion: Increased vigilance, bed pan and urinal rounds and accurate identification of high-risk patients will help to reduce inpatients' fall rate.

II023/CPIP

Reducing Blood Specimen Rejection by Laboratory

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Aim: TTSH laboratory rejects an average of more than 900 specimens per month, 2/3 of these are not rectifiable (e.g. specimen clotted) while the other 1/3 is rectifiable (e.g. wrong request form). This translates to at least 21 patients having to suffer a second venepuncture unnecessarily, not to mention the extra work for clerks, nurses, doctors and laboratory staff. TTSH's specimen rejection rate ranges from 0.26% (clinic with high volume) to 2.67% (ward with high volume) with a mean of 1.43%. The international standard for Haematology is 0.45% rejection out of 7.8 million specimens and for Biochemistry 0.35% rejection out of 10.7 million specimens. The team aimed to reduce laboratory rejection rate of level 8 blood specimens to less than 1% within 6 months.

Methods: We implemented the following interventions: 1) distributed a Tube Guide and Chart with details on handling of tubes and specimens for common and specialty specific tests to all clinical areas; 2) conducted customised training for level 8 doctors, phlebotomists, nurses and clerks.

Results: The specimen rejection rate of level 8 improved from 2.1% (July 2003) to 1.4% (December 2003), then to 1.2% (March 2004). The consumables and staff time saved translated into a tangible cost savings of \$3924 per year for level 8. The intangible saving of patient's "precious" blood, minimising specimen re-draw and avoiding delay in results are also equally important.

Conclusion: Regular training and an easy to use tube guide and chart can result in cost savings and improved patient satisfaction. In addition, wider availability of phlebotomists will further improve specimen rejection rate. This approach can be easily spread to all locations.

II024/CPIP

Improvement of INR Target Process Capability for Warfarinised Patients in the RAI Department

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Aim: The INR target process capability (TPC) of our department was running at an average of 58% over the 1-year period of 2002. The aim of this project is to improve the INR TPC for warfarinised patients in our department to 90% in 6 months.

Methods: Using the Clinical Process Improvement Programme (CPIP) methodology, a committee consisting of 2 nurse clinicians, a doctor, a pharmacist, a counter staff and a patient brainstormed the causes for poor INR TPC amongst our patients. The 3 top reasons were (1) poor knowledge of patients, (2) inadequate feedback about patient's understanding and (3) excessive tampering of warfarin dosage. 2 process changes were initiated: (1) patient education was done using standard materials, guided by a checklist, with re-enforcement of knowledge using a pre/post education assessment; (2) doctors were asked to titrate INR using a standard protocol. The project was implemented in the whole department from August 2003 to January 2004.

Results: The INR TPC of patients 12 months before implementation of the process changes was averaging at 58%. After the implementation, the average TPC was 83% over a 5-month period. There was no hospitalisation of patients due to under or over-anticoagulation during the implementation of the new processes.

Conclusion: Significant improvement was achieved for the INR TPC of warfarinised patients in our department using the CPIP methodology. Continuous measurement of INR TPC is currently undertaken and new PDSA cycles are being looked into to improve other areas that affect the INR TPC.

II025/MPC

A New Pressure Half Constant to Estimate Prosthetic Mitral Valve Area

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Aim: The pressure halftime (PHT) method is widely used to estimate mitral valve area (MVA) in patients with mitral stenosis. However, it is believed to be inaccurate in patients with prosthetic mitral valve. We wish to identify a new constant that will accurately estimate the prosthetic MVA using the PHT method.

Methods: We measured the MVA using PHT in 55 patients with a bileaflet prosthetic mitral valve. This was compared with MVA obtained using continuity equation (MVA-CE). The first 20 patients served as the derivation cohort. A new PHT constant was obtained using the formula (MVA-CE) x (PHT). The accuracy of the new PHT constant was then validated in a second cohort of 35 studies. Bland-Altman plots were used to compare the difference in means of the 2 tests.

Results: A new PHT constant of 190 was derived from the first 20 patients. Using the new constant in the subsequent 35 studies, we found a good agreement between MVA-PHT and MVA-CE across a wide range of mean MVAs, with near equal distribution above and below zero and most values (97%) falling within 2 Standard Deviations (SD) of the difference in mean MVA.

Conclusion: MVA when estimated using the new PHT constant of 190 is in good agreement with MVA estimated by CE. This new PHT constant should be used to estimate the MVA of a bileaflet mitral prosthesis.

II026/MPC

The Difference in Length of Stay in Isolation among Suspect TB Patients Admitted to TTSH and CDC2 after Introduction of Weekend AFB Smears

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Aim: To study the difference in length of stay (LOS) in isolation for suspect TB patients admitted to TTSH and CDC 2, before and after 17 January 2004, when weekend AFB smears were introduced.

Methods: Isolated patients admitted from November 2003 to March 2004 were included. We used the multivariate linear regression model to study the difference in length of stay in isolation (LOSI) between those admitted before and after 17 January 2004. LOSI was transformed and analysed on the natural logarithmic scale. Data was analysed in Stata (v7.0).

Results: 343 admissions were analysed. The mean LOSI was 5.0 ($\pm$ 5.0) days, with a median of 4 days (IQR: 3-6 days). Mean age was 62.4 $\pm$ 20.0 years, with most of them, 231 (67.5%) being male. Eighty-six (25%) were admitted on a weekend. The overall group of patients have no significant difference in LOSI before and after 17 January 2004 but suspect TB patients admitted on weekends after 17 January stayed on average 1 day less.

Conclusion: TB suspect patients admitted to TTSH and CDC2 wards on weekends after 17 January 2004 stayed on average 1 day less than those admitted before the date in spite of the fact that there was no overall change in length of stay amongst cases admitted to isolation rooms over the period.

II027/MPC

Role of Nuclear Imaging in Sports Medicine

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Aim: Nuclear imaging using single-photon-emission tomography (SPECT) and 3-phase bone scan is a well-established, highly effective diagnostic tool. Although many conditions can be diagnosed with a good history and physical examination, specialised imaging techniques may be needed to confirm or exclude a serious disorder. The diagnosis of stress-related musculoskeletal disorders still constitute the most popular application of radio-nuclide imaging in sports medicine. These conditions include stress fractures, avulsion fractures, periostitis ("shin splint"), myositis ossificans, rhabdomyolysis, bony contusion and avascular necrosis.

Methods: It involved a retrospective study of related cases performed in the

Nuclear Medicine Laboratory, Department of Diagnostic Imaging, NUH.

Results: We present a poster review of common sports-related musculoskeletal disorders that were diagnosed on Nuclear Medicine scans.

Conclusion: Bone scintigraphy is a highly sensitive, widely available and relatively inexpensive method for diagnosing many stress-related skeletal injuries. The greatest strength of the radio-nuclide scan relates to its ability to provide early physiologic information about the involved organ system and to evaluate multiple areas in a single, relatively rapid examination. Improved imaging techniques such as SPECT and 3-phase scanning, together with the recognition of scintigraphic patterns, have improved scintigraphy's diagnostic specificity for many sports-related injuries.

II028/MPC

Multidetector CT Pulmonary Angiography in Clinically Suspected Pulmonary Embolism

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Aim: To describe our experience with multi-detector CT pulmonary angiography in patients suspected of having pulmonary embolism (PE).

Methods: Retrospective analysis of data of patients who underwent CT pulmonary angiography (CTPA) for clinically suspected pulmonary embolism was done. The study included unselected patients who underwent CTPA during a 36-month period. A total of 524 patients underwent CTPA. The follow up period was taken till the last date of visit to the hospital by the patient at the time of data collection or death. The follow up period ranged from 1 to 903 days (mean 197.3 days). D-Dimer, Doppler tests and ventilation-perfusion scans were done in 334, 155 and 13 patients, respectively.

Results: CTPA was positive for PE in 107 patients and negative in 416 patients. There was one false negative scan. In one patient, the study was indeterminate. A total of 146 patients received anticoagulation treatment. Among these, 5 patients were treated with anticoagulation due to high clinical suspicion. At the end of the follow up period, there were 121 deaths (10 due to PE). Among the negative CTPA patients, there was no PE or PE related mortality during follow up. The overall sensitivity and specificity of CTPA in detection of PE was 99.07% and 98.6%, respectively. Alternate diagnosis could be demonstrated in nearly 80% of patients with negative CTPA.

Conclusion: Multi-detector CTPA has good sensitivity and specificity for diagnosis of PE. In majority of patients who do not have PE, it demonstrates alternate findings for final diagnosis.

II029/MPC

High Resolution Computed Tomography of the Lung Detection of Air Trapping Due to Small Airways Disease Using Ultrafast Dynamic Scans During Forced Expiration

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Aim: The use of high resolution computed tomography (HRCT) in the diagnosis of small airways disease has been well documented. Inspiratory and expiratory HRCT of the lungs can identify affected airways that are conducting to areas of air trapping. In patients with mild disease, lung function tests may be negative or equivocal. In these instances, HRCT of the lungs, with additional dynamic ultrafast scans during forced expiration can be of use to identify subtle areas of air trapping.

Methods: We reviewed HRCT studies done at our institution over a period of 6 months. Those scans with additional dynamic expiratory scans were reviewed to identify areas of air trapping. Correlation of the HRCT findings with clinical diagnosis and lung function tests was made.

Results: In patients who underwent additional dynamic expiratory scans during HRCT study, the finding of air trapping was useful in the diagnosis of small airways disease, especially where lung function tests were normal or equivocal. The additional scans were helpful in identifying air trapping, compared with simple post-expiratory HRCT.

Conclusion: Air trapping as an indicator of small airways disease can be identified and quantified using HRCT. Ultrafast dynamic expiratory HRCT can be a useful adjunct in identifying subtle air trapping in patients with small airways disease.

II030/MPC

Gender Differences in Hippocampal Volume in Patients with Psychosis

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Aim: To examine the hippocampal volume in minimally treated patients with first-episode psychosis as compared to normal controls and to look at the correlation between the hippocampal volume and clinical data.

Methods: From contiguous 1.5-mm coronal magnetic resonance images, the hippocampal formation was divided into posterior and anterior segments, and the anterior hippocampal formation was separated from the amygdala. Volumes of bilateral, posterior and anterior hippocampal formation were computed in 27 patients and 16 healthy controls matched for age, gender and handedness.

Results: Compared to controls, patients had statistically significant smaller mean right hippocampal and right anterior hippocampal volumes. There was a significant gender-by-diagnosis-by-hemisphere interaction for hippocampal volume. Hippocampal volume on the right was significantly smaller in female patients than in female controls. Male patients and male controls demonstrated no significant difference in hippocampal volume. There was no significant correlation between hippocampal volume and duration of untreated psychosis as well as psychopathology as measured by the Positive and Negative Scale for Schizophrenia (PANSS).

Conclusion: These findings suggest that there may be a unique interaction between gender and the disease processes that lead to reductions in hippocampal volume in patients with schizophrenia.

II031/MPC

Management of Acute Pulmonary Oedema with High Dose Intravenous Glyceryl Trinitrate

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Aim: A starting dose of intravenous (IV) glyceryl trinitrate (GTN) at 10 to 20 µg/min is recommended for management of acute fluid overload, i.e. acute pulmonary oedema (APO). This study describes the use of high dose (>20 µg/min) IV GTN in APO management in the Emergency Department (ED).

Methods: The ED computer system was searched from September 2003 to April 2004 for patients with APO. Eleven patients received high dose IV GTN and a retrospective chart review was done for them.

Results: There were 5 men and 6 women, with a mean age of 69.7 years. The mean systolic blood pressure (SBP) on arrival was 204.9 mmHg, heart rate was 107.7/min, SaO₂ was 94.2%. The mean dose of frusemide was 123.7 mg and morphine was 3.5 mg, with 3 patients not given morphine. The mean starting dose of IV GTN was 21.4 µg/min, with 7 patients given an initial 1 mg bolus. The mean SBP on leaving ED was 151.8 mmHg. The mean length of stay in ED was 1.45 hours. Two patients required intubation in ED and 1 patient was intubated subsequently in CCU. Seven were admitted to the general ward and 4 to CCU. Except for one patient diagnosed to have pneumonia, ED diagnoses of APO matched discharge diagnoses in the other patients: one was due to a renal cause while the rest had cardiac causes. All patients were discharged alive with mean length of stay of 5.1 days.

Conclusion: High starting dose of IV GTN appeared safe and efficacious in APO management.

II032/MPC

Elderly in an Emergency Department

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Aim: The study aims to examine the use of emergency service by the elderly in an emergency department (ED) in Singapore and its outcomes.

Methods: Patients aged 65 years and above who attended the emergency department at National University Hospital from 18 February 2002 to 31 March 2002 were retrospectively studied. Data was collected from NUH EMD and HIDS records.

Results: The elderly (aged 65 years and above) represented 1270 (15.6%) out of the total 8216 ED attendances for all adults (aged 16 years and above) from 18 February 2002 to 31 March 2002. The mean age was 75.3 years old with ages ranging from 65 to 107 years. 35% of them arrived via ambulance; 1169 (92%) were emergencies with non-traumatic causes forming the majority (85%). 72.3% of the patients were admitted.

Conclusion: Though the elderly formed only 15% of the total ED attendances, it constituted 34.7% of total ED admissions. Understanding the use of emergency services by the elderly and its outcome is important as the elderly population has their own distinct physical, physiological and social needs. As the elderly population increases, the information will aid us in the evaluation and disposition of the elderly.

II033/MPC

The ED Heartache

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Aim: The emergency management of patients with suspected ACS has been the subject of numerous studies. An ideal, safe, yet efficient approach for managing chest pain patients presenting to the emergency department has yet to be found. The aim of this study was to describe the current state of management of chest pain patients at a tertiary teaching hospital.

Methods: Retrospective audit: Patient admission and diagnosis data were retrieved from the hospital medical information services (MIS) over a period of 6 months from January 2003 to June 2003. Chest pain patients were identified from ICD codes and their dispositions reviewed.

Results: Of 1427 chest pain patients identified during the study period, 13% were diagnosed to have acute myocardial infarction (AMI) at the ED and the other 87% diagnosed to have angina or chest pain. 56% of the patients were admitted, while 40% were discharged. The remainder were either transferred or discharged against advice. Of patients admitted to the wards, 39% were discharged with a diagnosis of "chest pain", and 21% had non cardiac diagnoses. Another 12% were found to have AMI after admission. 26% patients had a length of stay of 1 day, and another 26% stayed 2 days.

Conclusion: This study has identified some areas for improvement and forms a historical database for future studies in the continuing search for a safe yet efficient approach to the management of patients with chest pain.

II034/MPC

Are Homicide Offenders Psychiatrically Different from Offenders of Other Violent Crimes?

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Aim: The association between mental illness and violent offenders is an important issue. Several studies have found an increased prevalence of psychiatric illness among offenders. This study seeks to update the psychiatric community on individuals charged with murder or voluntarily causing hurt (VCH) from 1997 to 2001, the last major psychiatric homicide study here being in 1985.

Methods: Fifty-three homicide offenders and 80 controls had psychiatric diagnosis(es). Demographic data, diagnoses, offence and victim profiles were compared, with analyses done using SPSS.

Results: Demographically, the 2 groups were similar. A positive history of violence was significantly less likely in homicide offenders, as was schizophrenia. Alcohol and substance abuse or dependence and depression were reported with greater frequency in the homicide group. Of those with psychotic disorders, persecutory delusions were found more often with the homicide offender group. The use of dual methods of sharp trauma and blunt trauma was employed significantly more by the homicide offenders.

Conclusion: While not totally preventable, reduction of the rate of homicide in the country may be achievable via the reduction of controllable factors found to be linked to the aetiology of murder. In this study, use of alcohol and other illicit substances is frequently found to be in association with homicide. In Singapore, campaigns to dissuade alcohol abuse have not been as prominent

as other health prevention programmes, such as those on smoking cessation. The authorities are urged to strongly consider implementing such programmes.

II035/MPC

Migration Psychosis and Homicide in Singapore

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Aim: Studies have found increased incidence of psychotic and mood disorders in migrant populations. Further, a higher percentage of immigrants has been found in schizophrenic murderers compared with crime-free schizophrenics. This study aims to determine if "migration psychosis" is valid in Singapore homicides. International literature also addresses under-utilisation of psychiatric services by some migrant populations.

Methods: Foreigner and local homicide offenders from 1997 to 2001 were compared using SPSS with regard to demographic data, psychiatric diagnoses, offence and victim profiles.

Results: Thirty homicide offenders were foreigners. The proportion of foreigner homicide offenders was significantly higher than the proportion of foreigners in the population. Foreigners who commit homicide also appear to suffer from the more serious psychiatric illnesses of mood and psychotic disorders compared with the locals, but yet are less likely to be known to have had a history of violence or a past forensic history. The foreigner homicide offenders were more likely to be new to psychiatric services compared with the local offenders and thus here, be presenting to a psychiatrist only after they have killed another person.

Conclusion: Foreigners appear to be at higher risk of developing a serious mental illness, not being treated for it and then committing an act as tragic as murder. A simple programme for educating employers and newly recruited foreign workers may curb such happenings. Basic orientation to the spectrum of healthcare resources available, including psychiatric services, would alleviate some of the stresses of being in a strange land.

II036/MPC

Epidemiology of Patients with Sepsis Admitted to a Medical Intensive Care Unit in Singapore Over 3 Months

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Aim: Critically ill patients with sepsis are known to have high mortality rates. However, there is no published data on the epidemiology of these patients presenting to the Medical Intensive Care Unit (MICU) in Singapore.

Methods: This prospective, observational study investigates the clinical presentation and outcome of patients with sepsis who were admitted to the MICU of a local university-affiliated hospital over 3 months (March to May 2004).

Results: Over the period of study, there were 133 new cases admitted to the MICU, of which 41 (30.8%) were patients with sepsis. Nine of them were female (22%). The mean age $\pm$ SD was 61.2 ± 14.5 years (median: 63; range: 30-82). Only 4 patients were admitted directly from the Emergency Department. The most common primary source of infection was from the lungs (82.9%). Three patients were admitted with hospital acquired infections. The mean APACHE II score was 30.6 ± 7.5 (median 30; range 17 to 48). The average ICU length of stay was 8.6 ± 7.7 days (median 6; range 1 to 32) and the ICU mortality rate was 41.5%.

Conclusion: The ICU mortality rate of our study population was 41.5%. This is within the range of other published epidemiological studies of critically ill patients with sepsis in the ICU.

II037/MPC

Outcomes of Patients Referred to a Specialised Falls and Balance Clinic

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Aim: To determine the outcomes of patients referred to a specialised Falls and Balance Clinic and factors affecting these outcomes.

Methods: Retrospective chart review of 193 patients (mean age $\pm$ standard deviation: 78.1 years $\pm$ 7.6 years) referred to a specialised Falls and Balance Clinic over a 1-year period. Demographic data and clinical parameters were systematically extracted for analysis.

Results: Seventy-one patients (36.8%) remained in the Falls and Balance Clinic programme for a minimum of 6 months. Seventy-eight patients (40.4%) were discharged from the clinic prior to completing the 6 months programme. Forty patients (20.7%) defaulted follow-up and 4 patients (2.1%) died during the 6-month follow-up period. Of the group of patients who remained in the programme for 6 months, 70.0% reported that they had benefited from the interventions in terms of increased confidence in mobility. 47.2% and 64.0% of patients had improvement in the timed 'Up and Go' test and Berg Balance Scale score respectively. On multivariate analysis, compliance to physical therapy was significantly associated with subjective improvement ($P = 0.021$).

Conclusion: The findings indicate that the majority of patients who adhered to a specialised Falls and Balance Clinic programme do benefit in terms of increased confidence in mobility. The beneficial effect of the programme is dependent on compliance to physical therapy. This highlights the need to educate patients and their carers on the importance of adhering to a falls prevention programme.

II038/MPC

The Use of a Behavioural Pain Rating Scale in Nursing Home Residents with Cognitive Impairment

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Aim: To assess the construct validity and the diagnostic accuracy of the Pain Assessment in Advance Dementia (PAINAD) scale in assessing pain.

Methods: Design: Case control design. Participants: Ninety-nine nursing home residents with moderately severe cognitive impairment. Measurements: Residents were asked to recall their average pain over 1 week. Nurses scored the PAINAD scale, a behavioural pain scale, by recalling the type of pain behaviours the resident exhibited in that same week. Residents were also assessed with the Cornell's scale of depression in dementia (CSDD) and the Abbreviated Mental Test (AMT) score.

Results: There was a significant correlation between the PAINAD score with the SRPS (spearman's rho [sr] = 0.363) and with the CSDD score (sr = 0.350), but not with the AMT score. Patients with and without significant depressive behaviours (CSDD score of 8 or more) had statistically different PAINAD scores but not SRPS. There was no statistical difference in PAINAD scores and SRPS among patients with decreasing AMT scores. For residents without depression, the following scores had a modest relationship with the SRPS (0 = no pain; 1-2 = mild pain; 3 and above = moderate pain and above) (sr = 0.526). For residents with depression, the following scores had a low relationship (0-1 = no pain, 2-3 = mild pain; 4 and above = moderate pain and above) (sr = 0.332).

Conclusion: The PAINAD has a modest relationship with the SRPS in nursing home residents without depression, but a low relationship in residents with depression.

II039/MPC

Caregiving in Dementia

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Aim: Caregiving in dementia is a demanding task and the quality of life of the patient is affected much by the quality of care he receives. As such, understanding the caregiver is important as his knowledge, beliefs and attitudes towards dementia and caregiving would impact the quality of care he provides.

Methods: Using a standard questionnaire, we studied caregivers with respect to their approach towards certain problems in caregiving, their perception of how dementia has affected the relationship between the patient and themselves and administered the Short Zarit Burden Interview to assess caregiver burden.

Results: Out of more than 50 respondents in this ongoing study, more than

50% showed high burden scores and a similar proportion had difficulty finding meaning or deriving pleasure in caregiving. Caregivers also expressed difficulty in empathising with the patient and some even felt that the patient often behaved in a difficult way and irritated their caregivers on purpose.

Conclusion: The results suggest the need to help caregivers better understand the person with dementia. Person centered approaches to caregiving should be emphasised and finally, we need to help caregivers find meaning in caregiving and this could translate to lower caregiver burden.

II040/MPC

Concern for Family Members is a Strong Motivator for Genetic Testing in Patients at Risk for Hereditary Cancer Syndromes

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Aim: Cancer genetics clinics that evaluate patients for hereditary cancer syndromes are now part of routine oncology services. We prospectively studied the acceptance rate of genetic testing in high-risk cancer patients as there is limited data on this for adult-onset diseases in Asia.

Methods: High-risk cancer patients with at least 10% probability of carrying a genetic mutation for hereditary cancer syndrome were surveyed immediately after genetic counselling.

Results: Thirty-nine high-risk cancer patients, age ranging from 26 to 72 (median 37), participated, of which 85% and 15% were suspected to have hereditary breast or hereditary colorectal cancer, respectively. 79% were Chinese and 21% Malay; 92% were female, 77% were married, and 72% had children. 65% indicated interest to undergo genetic testing, 15% were uncertain and 20% declined. The major motivator was to gain information to help family members (50%), while the major reasons against were worries that testing would cause stress/anxiety/depression (13%), concerns about employability or insurability (13%), and perception that testing cannot prevent another cancer or recurrence (10%). Seventeen of the 26 patients (65%) who indicated initial interest in testing were actually tested. 3/26 decided against testing, and 6/26 needed more time to consider. 2/6 patients who initially said "no" to testing were subsequently tested.

Conclusion: Interest and actual uptake in genetic testing for hereditary cancer syndromes among Singaporean high-risk cancer patients is high, and the major motivator is to gain information to help family members.

II041/MPC

Clearance of Anticancer Drug Docetaxel is Strongly Inhibited by the Antifungal Agent Ketoconazole, Which is a CYP3A Inhibitor

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Aim: We previously reported CYP3A phenotyping with midazolam predicted interindividual variations in docetaxel pharmacokinetics (PK) [J Clin Oncol 2002;20:3683]. This study investigated if CYP3A inhibition by ketoconazole could reduce interindividual variability in docetaxel clearance, thereby allowing safe dosage standardisation, and whether CYP3A phenotyping could predict this inhibition.

Methods: A dose escalation study was conducted using docetaxel infusion q3w in combination with per oral ketoconazole 2 days before and after docetaxel. The pharmacokinetics of docetaxel and IV midazolam 1 mg in each dosage group were studied using non-compartmental analysis.

Results: Twenty-seven patients with tumours refractory to standard chemotherapy were enrolled at docetaxel doses of 10 (2 patients), 20 (6 patients), 25 (7 patients), 30 (6 patients) mg/m² and 50 mg (6 patients) standard dose; 2 were unevaluable. Ketoconazole did not reduce docetaxel clearance variability across and within dose levels. Docetaxel clearance was 5.88 ± 2.69 , 9.83 ± 4.64 , 8.07 ± 2.78 and 8.21 ± 2.86 L/h/m² at doses of 20, 25, 30 mg/m² and 50 mg respectively. Mean midazolam clearance was 4.69 ± 2.1 L/h, 5.3-fold slower with ketoconazole, compared to our previous data. Mean docetaxel clearance was 2-fold slower, 7.73 ± 3.66 L/h/m². Midazolam clearance ($r = 0.29$, $P = 0.18$) did not correlate with docetaxel clearance.

Conclusion: Ketoconazole strongly inhibits midazolam and docetaxel

clearances by unequal extent and does not reduce variability for docetaxel. This has strong implications for drug interactions and possible application in cost savings for docetaxel treatment.

II042/MPC

A Comparison of Tuberculin Skin Testing Using 1 TU RT 23 PPD Versus 2 TU RT 23 PPD in Healthy TB Contacts

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Aim: In Singapore, historically 1 unit RT 23 PPD (equivalent to 2.5 TU) has been employed for tuberculin skin test (TST). In the US and several other countries, 5 TU is used (equivalent to 2 unit RT 23 PPD). This makes it difficult to compare the rates of TST positivity with other countries. We did a prospective randomised study to assess the difference in TST reading for 1 versus 2 units RT 23 PPD.

Methods: Healthy contacts of TB patients who came for routine TST were consecutively enrolled after informed consent. 2 TSTs were performed simultaneously in each subject using 1 and 2 units RT 23 PPD (Serum Statens Institute, SSI). Each dose was randomly assigned in a blinded manner to the right or left forearm and read at 48-72 hours by experienced nurses who were blinded to the assignment.

Results: A total of 79 subjects (71 of whom had a history of past BCG vaccination) were enrolled. The overall mean for reaction size for 1 TU was 7.15 mm and for 2 TU 8.13 mm. The overall mean difference was 1.96 mm, and this difference was significant ($P < 0.001$).

Conclusion: The mean difference of 1.96 mm should be considered when comparing rates of TST positivity between countries that use different doses of tuberculin. This difference may have less impact in countries with high rates of BCG coverage and smear positive cases.

II043/MPC

Predictors of Lower Limb Amputations in Patients with Diabetes Mellitus

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Aim: To identify the predictors of amputation in patients with diabetes mellitus.

Methods: A retrospective study was planned on 140 patients with diabetes mellitus (DM) who underwent lower limb amputations in a general hospital in Singapore, between 2001 and 2003. We report results of the first 19 patients. Case records with procedure coding for amputations were retrieved for the predictors of lower limb amputations. Parameters analysed were HbA1C, lipid status and blood pressure on admission. Duration of DM, precipitating factors for the lower limb ulcers, location of the ulcers and outcome following amputations were also recorded.

Results: Ten were males and 9 females. Thirteen patients (68%) underwent "Below Knee Amputation" and 9 had Ray's amputation (32%). All patients had infection of lower limb as the precipitating factor for amputation. Six had ulcers in the toes, 7 had ulcers in metatarsal region and 4 in the heel. Hypertension and hyperlipidemia were the most common risk factors, involving 63%. Pre-existent atherosclerotic disease was also an important predictor. Poor glycemic control was seen in 63%. Nine patients had Ankle/Brachial index < 0.9 (47%). Eleven out of 12 (92%) patients had neuropathy as evidenced by sensory deficit and/or absent ankle jerk. Nine patients were either smokers or ex-smokers. Outcome of the amputations was that 10 patients were ambulant while 3 became bedbound, 4 became wheelchairbound while 1 patient died.

Conclusion: Hypertension, hyperlipidaemia, peripheral artery disease and neuropathy were significant predictors of amputation in a diabetic cohort.

II044/MPC

Outcome from Molecular Adsorbents Recirculating System (MARS™) Liver Dialysis Following Drug-induced Liver Failure

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Aim: Fulminant liver failure from drug ingestion is associated with a high mortality, and the introduction of liver transplantation has improved the mortality significantly if done in a timely fashion. Recently, MARS™ liver dialysis has been introduced as a support for liver failure with varying results. We review our experience with drug-induced liver failure and the impact of MARS™ liver dialysis on the outcome, in a setting where cadaveric liver transplantation is rarely available.

Methods: A total of 13 patients were treated, and 40 sessions of MARS™ liver dialysis were conducted in the intensive care unit. The majority of cases were due to herbal medicine toxicity.

Results: Total bilirubin and conjugated bilirubin were significantly reduced, with no change in unconjugated or delta bilirubin. All patients satisfied the criteria for urgent liver transplantation with an average MELD score of 35. Only one patient received a liver transplantation from a live donor (right lobe). Overall mortality was 85%. Median time to death from the start of MARS™ was 8 days.

Conclusion: MARS™ liver dialysis in a setting without timely liver transplantation is associated with a poor outcome. It does, however, provide a window of time for consideration of living donors in the setting of limited cadaveric donors.

II045/MPC

Tilt Table and Autonomic Testing: Review of Indications for Referrals and Results

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Aim: Tilt table and autonomic testing can be a useful supplementary test for differentiating the cause of loss of consciousness, orthostatic symptoms and some non-specific symptoms. We set out to establish the indications for referrals to our tertiary care tilt table and autonomic testing and the rate of corresponding abnormal results.

Methods: Retrospective review of all indications and results of patients referred for tilt table (blood pressure responses) and autonomic testing (heart rate variability) in the year 2002 at the Department of Medicine, Neurology Diagnostic Laboratory, National University Hospital, Singapore.

Results: Eighty-nine patients were tested (51 females, average age 49; range 17-86 yrs). The 4 major causes for referral and the corresponding number of abnormal results respectively were non-specific giddiness ($n = 42$; 20); single episode of loss of consciousness ($n = 33$; 10); recurrent loss of consciousness ($n = 6$; 4); orthostatic hypotension ($n = 5$; 4).

Conclusion: The most common indication for referral for tilt table and autonomic testing was for non-specific giddiness followed by a single episode of loss of consciousness. Referrals for recurrent loss of consciousness and orthostatic hypotension though much less common showed very high rates of abnormal test results. In our setting, screening tests seem useful in helping to establish or exclude a blood pressure regulatory or autonomic cause of symptoms.

II046/MPC

Blood Pressure Control in Patients with Chronic Renal Failure

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Aim: The JNC-VII recommends target blood pressure of $< 130/80$ mmHg for patients with diabetes and chronic renal failure (CRF). Recent studies suggest that blood pressure control in patients with CRF was still suboptimal even in clinics that have specialised in the treatment of hypertension.

Methods: We conducted a retrospective analysis of blood pressure control in patients with CRF who were followed at Tan Tock Seng Hospital between January 2004 and May 2004. Patients were included in the analysis if they had serum creatinine between 110 and 400 $\mu\text{mol/L}$ and they had been followed up in our outpatient renal clinics for at least 3 months. A total of 190 patients (age 22-87, mean 67) were included in this analysis. 61% of the patients were diabetic. Blood pressure measurement during office visit, the most recent serum creatinine, and current antihypertensive agents were retrieved from the patient's medical records.

Results: 31.6% and 46.3% of the patients were able to achieve target systolic blood pressure (SBP) of < 130 mmHg and diastolic blood pressure (DBP) of

<80 mmHg, respectively. On average, patients took 2.48 antihypertensive drugs. The most common prescribed antihypertensive drug was ACE-inhibitor and angiotensin receptor blocker (69.5%), followed by diuretics (64.2%), beta blocker (53.2%), and calcium channel blocker (36.8%).

Conclusion: In patients with CRF, achieving target SBP is more difficult than achieving target DBP. Less than half of the patients with CRF were able to achieve target blood pressure of <130/80 mmHg.

II047/MPC

Use of Angiotensin Converting Enzyme Inhibitors (ACE-I)/Angiotensin Receptor Blockers (ARB) in Patients with Diabetic Nephropathy and/or Proteinuria

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Aim: Studies have shown beneficial effects of ACE-I/ARB in retarding the progression of DM nephropathy, as well as in the reduction of proteinuria.

Methods: We conducted a retrospective analysis of patients who were followed up at the outpatient renal clinics of Tan Tock Seng Hospital between January and May 2004. Patients were included if they had diabetes with a serum creatinine of between 100 and 400 $\mu\text{mol/L}$, and/or if there was proteinuria (secondary to diabetes or otherwise) of more than 300 mg/day with a serum creatinine of less than 400 $\mu\text{mol/L}$. The patients must have been followed up for at least 3 months. The current medications were retrieved from the patients' medical records.

Results: Of the 115 diabetic patients who had a serum creatinine of 100 to 400 $\mu\text{mol/L}$, 69.6% were prescribed an ACE-I or ARB, 4.3% were prescribed both ACE-I and ARB. Of the 174 proteinuric patients who had a serum creatinine of less than 400 $\mu\text{mol/L}$, 78.2% were prescribed an ACE-I or ARB and 5.2% were prescribed both ACE-I and ARB. Reasons for not prescribing ACE-I/ARB were mainly hyperkalaemia, significant worsening of creatinine after initiation of ACE-I/ARB or pre-end stage renal disease.

Conclusion: Most of the patients with diabetic nephropathy and/or proteinuria in our renal clinics were prescribed an ACE-I or ARB, as these medications have been shown to be beneficial in retarding the progression of diabetic nephropathy, as well as for its anti-proteinuric effects.

II048/MPC

The Prevalence of Malnutrition in Inpatients in the National University Hospital

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Aim: Malnutrition adversely affects patients' outcomes, including hospital stay. It is also potentially reversible. This study aimed to determine the prevalence of malnutrition in a population of patients admitted to hospital.

Methods: All patients admitted to a single ward in August 2003 (totalling 85) were studied. Mean age ($\pm$ SD): 55.9 $\pm$ 17.6 years; height ($\pm$ SD): 1.58 $\pm$ 0.09 metres; weight ($\pm$ SD): 60.5 $\pm$ 12.8 kg. 35% were males. 51.8% were Chinese, 28.2% Malays and 17.6% Indian. 53% were diabetics and 43% had end-stage renal failure (ESRF). The patients' diagnosis and co-morbidities were noted. The following indices of nutrition were: body mass index (BMI) calculated using the formula: dry weight (kg)/height x height (m^2). BMI >25: overweight and well-nourished. A revised Subjective Global Assessment (SGA) 7-point scale was used: SGA of 1-2 is severely malnourished; SGA of 3-5 is moderately malnourished; SGA $\geq$ 6 is well nourished. Fifty-five patients had their SGA and BMI assessed.

Results: Using the BMI, 5 patients (5.9%) were underweight and malnourished, and with SGA, 26 patients (47.3%) were malnourished. When compared with age, gender, ethnic group, diabetes and ESRF, only diabetes and ESRF were positively associated with a higher likelihood of malnutrition.

Conclusion: This study reveals that malnutrition is a major problem in inpatients. Malnutrition seems to be more frequently associated with diabetes as well as ESRF. BMI seems to be a less sensitive indicator of malnutrition.

II049/MPC

White Coat Hypertension in the Elderly

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Aim: The benefit of treating hypertension in the elderly has been well established. However, treating one with the white coat response can be detrimental, especially in this group already at risk for falls. This retrospective study was conducted to determine the prevalence of white coat response in elderly subjects and to emphasise the importance of correct diagnosis to reduce overtreatment.

Methods: Twenty-four hour ambulatory blood pressure (ABP) records of elderly patients 65 years old and above, performed from 2001 to 2003 in the general medicine clinic, were reviewed. The clinical records were retrieved, identifying the clinical blood pressure reading, the underlying clinical history and antihypertensive regimen used when the test was requested.

Results: Of the 286 24h ABP records reviewed, 45 elderly patients were identified, of which only 39 clinical records were available. The mean age of the group was 72, with 49% male and 87% Chinese. 87% had underlying hypertension, with 44% demonstrating white coat response. In the remaining who were not previously diagnosed with hypertension, 60% proved to have white coat hypertension. Within the white coat responders, the mean clinic BP was 177/85 mmHg ($\pm$ 18/16), the mean ABP 129/65 mmHg ($\pm$ 7/9) and the mean difference 50 mm Hg ($\pm$ 19).

Conclusion: The elderly patient commonly exhibits a white coat response even with an underlying history of hypertension. Decision to start or escalate antihypertensive medication should be made with caution. Use of 24h ABP monitoring and a "start low, go slow" strategy should be considered in clinical management.

II050/MPC

Review of Patients with Carbon Monoxide Poisoning Admitted to Tan Tock Seng Hospital

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Aim: Carbon monoxide poisoning (COP) is one of the leading causes of death from poisoning worldwide. There is no published study of COP in Singapore so far.

Methods: All COP cases admitted to Tan Tock Seng Hospital over 5 years from 1999 to 2003 were retrospectively reviewed. The diagnosis was based on a history of exposure to smoke and elevated levels of carboxyhaemoglobin (COHb). The causes, demographic data, clinical presentations, management and complications were analysed.

Results: There were 12 patients with COP. Their average age was 38.9 ($\pm$ 11.8) years, with male to female ratio of 3:1. Accidental COP (58.3%) was commoner than intentional (41.7%). The commonest cause of accidental COP was smoke inhalation from a faulty vehicle. Gas stove was the most preferred source for intentional poisoning. Presenting features were headache (83.3%), confusion (83.3%), coma (12.7%) and agitation (8.3%). The mean COHb level on admission was 35.9% ($\pm$ 13.55). All the patients had their COHb levels normalised within 24 hours of admission. All were treated with 100% oxygen. Two (16.7%) required intubation and another was treated with hyperbaric oxygen. Acute complications were globus pallidus infarction (16.6%), adult respiratory distress syndrome (8.3%) and myocardial ischaemia (8.3%). Most of the patients (91.7%) were discharged well from the hospital. One patient developed early parkinsonism after a follow up of 2 years. There was no death.

Conclusion: COP is relatively uncommon in Singapore. It has a low rate of short- and long-term complications.

II051/MPC

Waist Circumference – A Significant Predictor of Metabolic Syndrome in Obesity

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Aim: The study is aimed at retrospectively analysing the predictors of metabolic syndrome (MS) in a non-diabetic obese cohort in an Asian population.

Methods: Retrospective analysis of biochemical and clinical parameters was conducted on 401 consecutive personnel of an organisation referred to the

weight management clinic of a general hospital in Singapore. All subjects were male. Clinical parameters analysed were age, BMI, waist circumference (WC), blood pressure and waist-hip ratio (WHR). Biochemical parameters measured after overnight fast included lipids and glucose. Metabolic syndrome was defined according to National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) guidelines. Statistical analyses were performed by χ^2 and logistic regression with significant P value <0.05 using SPSS 12.0.

Results: According to ATP III criteria, 10% of the study population had MS. Logistic regression analysis showed that WC (OR 1.093; 1.028-1.162) and age (OR 1.084; 1.034-1.133) were the best predictors of MS while BMI and WHR were not significant predictors. An increase in 1 unit in WC resulted in 9.3% increased risk of MS (95% CI, 2.8-16.2) and increase in 1 year of age resulted in increased risk of 7.2% (95% CI, 3.4-11.1%).

Conclusion: WC is a better predictor of MS compared to BMI and WHR. Increasing degree of WC and higher age are associated with higher risk of MS in obesity.

II052/MPC

Linitis Plastica: A Case Series, Review and the Role of Endoscopic Ultrasound

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Aim: Linitis plastica is an aggressive infiltrating tumour consisting of high-grade tumour cells accompanied by a marked desmoplastic reaction that results in a rigid stomach resembling a leather bottle. Typical barium and conventional endoscopic finding are present in less than half the cases. We describe the use of endoscopic ultrasound (EUS) to augment the diagnostic accuracy of this often-missed malignancy.

Methods: We report 5 cases of linitis plastica from August 2002 to April 2004 where the use of EUS aided greatly in the management of the patient.

Results: All 5 patients had endoscopic finding suspicious but not diagnostic of linitis plastica, and gastric biopsy was only positive in 1 case. All 5 patients had typical sonographic finding of linitis plastica, helping us to arrive at the correct diagnosis. One patient had MALToma of the stomach and the use of EUS was helpful in monitoring the progress of the tumour and planning subsequent management.

Conclusion: Endoscopic ultrasound appears to be a useful adjunct in the diagnosis of linitis plastica.

II053/MPC

Serum Albumin is Positively Associated with Better Quality of Life in Patients with Metastatic Solid Organ Cancer

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Aim: Serum albumin is a marker of nutrition that is associated with increased morbidity and mortality among the elderly and terminally ill. The relationship between serum albumin and quality of life (QOL) has not been studied among patients with metastatic solid-organ cancer.

Methods: We performed a prospective cross-sectional cohort study among patients with metastatic solid-organ cancer admitted to The Cancer Institute, Singapore, from February to April 2004. QOL was assessed using the European Organisation for Research and Treatment of Cancer QLQ-C30 questionnaire. Serum albumin measured on admission were compared with the function scales (physical, emotional, cognitive, social, role and global health), symptom scales (fatigue, pain, nausea/vomiting), and 6 single items assessing symptoms and the financial impact of the disease.

Results: Eighty-seven patients recruited were of mean age 60 years (range 29-90) who had a primary gastrointestinal tract (stomach and colon) (37%), lung (24%) and breast (13%) cancers. The mean serum albumin was 33 mmol/dL (range 16-50). The physical and role function scales were significantly lower

in patients with low albumin. Physical role and cognitive function scales, and improving dyspnoea and fatigue were significantly associated with a rise in serum albumin. Hypoalbuminaemia (<40 mmol/dL) was found to be more common in older patients and did not differ according to the primary site of malignancy.

Conclusion: Serum albumin is positively associated with better quality of life among patients with advanced cancer who may benefit from early nutritional intervention.

II054/MPC

Incidence of Pneumothorax in Critically Ill Patients with Severe Acute Respiratory Syndrome (SARS)

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Aim: During the SARS outbreak in Singapore in 2003, Tan Tock Seng Hospital (TTSH) was designated as the national SARS hospital. 96.6% (199/206) of all probable SARS patients in Singapore were treated in our hospital. In this study, we analyse the incidence of pneumothorax in our SARS patients and whether its presence had any impact on morbidity and mortality.

Methods: Observational cohort study involving retrospective analysis of demographic, laboratory and radiological data. The patients were recruited from 1 March 2003 to 13 July 2003, when the last SARS patient was discharged from TTSH.

Results: 23% (46/206) of the probable SARS patients required ICU care. Pneumothorax was observed in 17.3% (8/46) of these ICU patients. Of these 8 patients with pneumothorax, 7 (87.5%) were on ventilatory support and 1 had spontaneous pneumothorax. The length of mechanical ventilation was significantly prolonged in patients who developed pneumothorax (median of 25 days compared to 15 days in those without pneumothorax). Mortality was 87.5% in patients who developed pneumothorax, compared to 12.5% in those without pneumothorax. Incidence of other complications in these SARS ICU patients were secondary pneumonia (52.2%), septicemia (34.9%), deep vein thrombosis (23.9%) and acute renal failure (19.6%).

Conclusion: Pneumothorax was the fifth commonest complication in our SARS ICU patients. Patients with pneumothorax had significantly increased days of mechanical ventilation and mortality.

II055/MPC

Serum Bilirubin is the Only Independent Variable Affecting Mortality on Liver Transplant Waiting List

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Aim: The MELD score has been shown to be the best predictor of short-term mortality on the liver transplant waiting list in the USA. We wanted to identify the factors affecting mortality on the liver transplant waiting list in Singapore, including the MELD score.

Methods: All patients who were listed on the liver transplant waiting list in Singapore from January 1997 to December 2003 were analysed. MELD was calculated according to the United Network for Organ Sharing formula. Univariate analysis was performed to identify factors affecting mortality on the waiting list and multivariate analysis by logistic regression. Categorical and continuous variables were compared with Chi square and Mann-Whitney U tests.

Results: There were 48 patients in the study. We found that on univariate analysis, bilirubin, INR, MELD score and Child's score significantly influenced mortality on the waiting list. However, on multivariate analysis, bilirubin was the only independent prognostic indicator of mortality on the waiting list (LR = 1.97 [95% CI: 1.08-3.61]). INR was found to be significantly correlated to bilirubin with Pearson's correlation $R = 0.63$; $P < 0.001$.

Conclusion: Bilirubin is the only independent factor affecting mortality on the liver transplant waiting list.

II056/MPC

Validity and Reliability of the PDQ-39 and the PDQ-8 in English-speaking Parkinson's Disease Patients in SingaporeLCS TAN¹, N LUO², M NAZRI², SC LI², J THUMBOO³¹Department of Neurology, National Neuroscience Institute, Singapore,²Department of Pharmacy, National University of Singapore, Singapore,³Department of Rheumatology, Allergy and Immunology, Singapore General Hospital, Singapore

Aim: The purpose of the study was to assess the validity and reliability of the Parkinson's Disease Health Related Quality of Life (HRQoL) Questionnaire (PDQ-39, UK English version, 39-point questionnaire) as well as its briefer version (the PDQ-8, 8-point questionnaire) among patients with Parkinson's Disease (PD) in Singapore.

Methods: Eighty-eight patients recruited from movement disorder clinics or patient support groups completed the PDQ-39 and EQ-5D, a generic HRQoL questionnaire (consisting of a classifier for 5 health dimensions and visual analogue scale) previously validated for use in Singapore.

Results: PDQ-39 items showed good convergent and discriminant validity. Construct validation against the EQ-5D showed strong correlation between these scales as hypothesised (Spearman's rho: 0.53 to 0.71, $P < 0.001$).

Conclusion: We conclude that the PDQ-39 and PDQ-8 are valid and reliable disease-specific HRQoL instruments for PD in Singapore.

II057/MPC

Appendectomy in Childhood Immunosuppression: In for a Rough Ride?SZAINUDIN¹, CH CHUI², MY CHAN³, Y LOW², TL YAP², A JACOBSEN²¹Department of Paediatrics, National University Hospital, Singapore,²Department of Paediatric Surgery, KK Women's & Children's Hospital, Singapore, ³Department of Paediatrics, KK Women's & Children's Hospital, Singapore

Aim: To identify clinical features which suggest poor prognosis in appendectomies performed in children with immunosuppressed states.

Methods: A retrospective review of demographic data, clinical presentation, radiological and laboratory investigations, operative and histological findings and postoperative outcome was conducted on consecutive immunosuppressed children who underwent appendectomy between January 2002 and February 2004.

Results: Ten consecutive patients with various forms of immunosuppression, median age 9.5 years, who underwent appendectomy for suspected appendicitis were reviewed. The underlying primary diseases were acute lymphoblastic leukaemia in 5, acute myeloid leukaemia in 2, aplastic anaemia in 2 and rhabdoid tumour of brain in 1. Except those with aplastic anaemia, all were receiving chemotherapy. Eight patients had neutropenia. The median duration of symptoms prior to diagnosis was 4 days (range, 1-7 days), with similar duration of intravenous antibiotics prior to surgery. All had CT scans (8 positive for appendicitis) done. Five had additional radiological findings of enterocolitis. Intraoperatively, there were 4 perforated appendicitis, 5 acute appendicitis and 1 normal appendix, confirmed on histology. There were 4 mortalities; all were neutropenic. Three of the 4 patients who were septicemic preoperatively died in the immediate postoperative period. All had stormy postoperative courses with multiorgan failure as end-point. The fourth death was unrelated to surgery.

Conclusion: Diagnosis of appendicitis is a challenge in this group of patients due to equivocal early clinical and radiological signs, differential diagnosis of neutropenic colitis and prior antibiotic therapy. Aggressive surgical management is suggested once appendicitis is diagnosed. Neutropenia and preoperative septicemia are possible indicators of poor prognosis.

II058/MPC

Osteosarcoma in a Paediatric and Young Adult Population in Singapore National University Hospital ExperienceL AUNG¹, A YEOH¹, PL TAN², TC QUAH¹, R PHO³¹Department of Paediatrics, National University of Singapore, Singapore,²Department of Paediatrics, National University Hospital, Singapore,³Department of Hand & Reconstructive Microsurgery, National University of Singapore, Singapore

Aim: More than 80% of children with osteosarcoma (OS) in the world relapse and 35% to 40% die within the first 2 years after diagnosis. There is limited information on survival regarding these tumours in the region of Southeast Asia. We thus attempted to investigate the incidence, the treatment modalities used and the outcome of OS in Singapore.

Methods: A comprehensive list of patients with OS treated at the Children's Medical Institute of the National University Hospital, Singapore between January 1997 and June 2004 was generated. During the study interval, patients received neoadjuvant chemotherapy followed by definitive surgery, consisting of either limb-salvage or amputation and adjuvant chemotherapy. Chemotherapy included combination of cisplatin and doxorubicin as per the European Osteosarcoma InterGroup (EOI).

Results: Of the 17 patients with OS, 7 patients presented with metastatic OS (lungs, $n = 5$; others, $n = 2$). The median age of diagnosis of OS was 11.6 years (range, 6.4-14.9). Median survival after diagnosis of OS was 1.4 years (range, 0.1-9.3). The approximate 2- and 5-year overall survival rates were 40% and 29%, respectively. At last follow-up, median 1.4 years, 7 of the 17 patients (40%) were dead of disease and 5 (29%) patients were alive with no evidence of disease. All except the 2 alive were treated on the EOI regimen.

Conclusion: Survival from OS in Singapore is poor compared to the rest of the world. The rarity and complexity of OS makes it crucial for patients diagnosed with this tumour to seek a specialised multi-disciplinary team approach. Further improvements in the chemotherapy regimen employed are necessitated.

II059/MPC

Rapid Diagnosis of Urinary Tract Infections in the Elderly

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Aim: Urinary tract infections (UTIs) in elderly patients are exceedingly common and account for substantial morbidity and economic costs. These infections can occur in the community or may be acquired in nursing homes and hospitals. UTIs may also lead to complications. Therefore, early detection with appropriate intervention can potentially reduce the morbidity and mortality associated with urinary tract infection.

Methods: At Tan Tock Seng Hospital, urine cultures constitute 24% of total cultures sent to the laboratory. Common aetiological agents encountered in these samples are *Escherichia coli*, *Klebsiella* species, yeast and *Enterococcus* species. These organisms make up 72% of total urine isolates. Conventionally, the turnaround time for positive urine cultures is 2 days. Since 2002, the turnaround time has been reduced to 1 day, using the morphology and rapid tests for the identification of the majority of isolates.

Results: Some of the tests used for the identification of lactose-fermenting Gram-negative bacilli include spot indole, rapid MUG, motility, ornithine and lysine decarboxylases, pyrrolidonyl peptidase and oxidative-fermentative glucose utilisation. Antibiotic susceptibility tests are set up in the morning and read after 6 hours of incubation. Most positive results could be sent out 1 day after receipt of specimen.

Conclusion: Providing results after 1 day allows the clinician to initiate specific therapy earlier, alleviating the patients' symptoms and probably reducing complications. Patients may also be switched to suitable oral options allowing earlier discharge, thus contributing to decreasing healthcare costs in this group of patients.

II060/MPC

The Problem with the Classification of Auras in Temporal Lobe EpilepsyDPK LOH¹, MR TRIMBLE²¹Department of Psychology, Institute of Mental Health/Woodbridge Hospital, Singapore, ²Raymond-Way Neuropsychiatry Unit, National Hospital for Neurology and Neurosurgery, London, United Kingdom

Aim: One main feature of temporal lobe epilepsy (TLE) is the reports of auras by patients. An aura is the brief subjective experience that frequently precedes the onset of clinical seizures. Taylor and Lochery (1987) opined that the more complex an aura is, for example déjà vu aura, the more it could be subjected to systematic simplification, especially by the recording clinician. Past studies have shown that auras have been classified according to some a

priori invented rule of thumb as constructed by clinicians or researchers. This study attempted to verify if the objective expectations of clinicians and researchers, as represented in aura classifications, matched patients' subjective reports of auras.

Methods: The pre-surgical aura descriptions of 114 patients with TLE were recorded and categorised in an aura classification, the Aura Tree, which was constructed by a team of experts belonging to the Raymond-Way Neuropsychiatry Unit, National Hospital for Neurology and Neurosurgery, and has been shown to have face validity. It classified auras into 2 big categories, Experiential and Physical, which are further divided into sub-categories. The aura data was coded into 'yes' or 'no' responses and categorised according to the Aura Tree classification.

Results: Factor analysis could not be performed as poor correlations between aura sensations meant that distinct groups of auras could not be statistically derived.

Conclusion: It was suggested that the expectations of clinicians and researchers might not match the subjective experiences of patients.

II061/MPC

The Relationship Between Auras and Psychopathology Following Temporal Lobectomy

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Aim: The impact of temporal lobectomy on the relationship between auras and psychopathology in temporal lobe epilepsy patients has not been well explored. Most studies have been cross-sectional in their examination. The aim of this study was to investigate the short-term as well as long-term effects on this relationship.

Methods: 137 patients participated in Time 1 (1 year follow-up), whereas 61 (44.5%) of these participated in Time 2 (3 years after Time 1). Pre-surgical and short-term follow-up information was collected via patients' case-notes. A questionnaire was constructed to collect long-term follow-up information, as based on patients' subjective experiences in the preceding year. Psychopathology was defined by mood and anxiety disorders.

Results: Chi-squared tests of independence and Fisher's Exact test were used to examine distributions of aura sensations, various combinations of auras, and no aura groups, against mood and anxiety disorders. Short-term outcome was analysed by comparing pre-surgical and Time 1 data, whereas long-term outcome was analysed by comparing pre-surgical and Time 2 data. Results revealed that the chance of patients with pre-surgical aura(s) experiencing mood and anxiety disorders at Time 1 and Time 2 was not significantly different from the chance of patients without pre-surgical aura(s).

Conclusion: This study observed trends that not only were different from past studies, but were difficult to analyse and interpret. It may suggest that the number of auras experienced may not be associated with the experiencing of psychopathology. The results could be attributed to a number of methodological concerns.

II062/MPC

Impact of a Quality Improvement Program on Radiation Oncologist Performance: Evaluation of the Cancer Institute (TCI) Model Integrating Continuing Medical Education, Clinical Quality Assurance and International Revalidation Activities

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Aim: Clinical quality improvement (QI) may result from quality assurance (QA), continuing medical education (CME) or revalidation/recertification activities. Our aim was to evaluate whether the refined TCI-Singapore QI model integrating CME, QA and international revalidation activities resulted in improved radiation oncologist (RO) practice.

Methods: The QI program incorporated QA (random case audit, simulation audit, reminders), CME (protocols, audit feedback/discussion, directed/ad hoc tutorials) and revalidation (the random audit targeted RANZCR

revalidation-audit criteria). Program evaluation compared audit scores (behaviour and performance) and adherence to TCI protocols between the first 6 months (T1: June-November 2003) and second 6 months (T2: December 2003-May 2004) of the integrated program.

Results: 167 and 136 charts were evaluated during T1 and T2. Physician behaviour significantly improved between time-periods (from 10.0 to 10.7 out of 11, $P < 0.0001$), as did performance (6.7 to 6.9 out of 7, $P = 0.07$). Of 303 patients, 68% were eligible for protocols. Significantly more eligible patients were treated according to protocols at T2 compared to T1 (85% vs 68%, $P = 0.005$). The QI program resulted in 53 actions generated, including CME talks targeting deficient knowledge (14 actions), protocol alterations (8), systematic changes to RO practice (13), QA checks (8), and remediating deficient management of individual patients audited (10, representing 3.3% of all patients).

Conclusion: The TCI QI model incorporating CME, QA and revalidation effectively improved RO behaviour and performance, as well as departmental protocol adherence. Additional benefits included identifying CME topics, systematic changes in RO practice and altering management of 3.3% of patients audited.

II063/MPC

Patient Choice of Radiotherapy Fractionation Schedule in the Palliation of Non-Small Cell Lung Cancer: Design and Validation of a Decision Board

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Aim: Randomised studies of palliative radiation for NSCLC show relative advantages and disadvantages of short versus long fractionation schedules. Our aim was to develop a visual aid to accommodate patient choice in the palliative treatment of NSCLC.

Methods: A decision board was developed based on the British MRC study of 17 Gy/2 fractions versus 39 Gy/13 fractions, with advantages and disadvantages of each schedule displayed using proportions, graphs and confidence levels. A prototype was designed after evaluating decision board literature, then discussed with radiation oncologists, nursing staff and patients. Revisions were made to obtain an acceptable final version. The final version was piloted in 15 patients to ensure validity.

Results: Of 15 patients enrolled in the pilot, median age was 65, 93% were males, 73% were Chinese. 47% of patients spoke English, and none had ECOG ≥ 3 . 60% of patients had chest pain or shortness of breath as the main presenting symptom. 47% of patients chose the shorter fractionation for reasons of convenience (100%) and cost of treatment (34%). 53% of patients chose 39Gy/13 fractions, for reasons of better local control (50%), survival advantage (37%), less psychological distress (38%) and better physical activity (25%). All reasons for fractionation choice were consistent with decision-board information, confirming validity. All patients were satisfied with involvement in decision-making.

Conclusion: We have validated a decision board assisting patient choice of radiotherapy fractionation schedules in the palliation of NSCLC. A phase II study evaluating patient preferences using the instrument is underway.

II064/MPC

Physical, Cognitive-Behavioural and Educational Group Interventions in Fibromyalgia: Preliminary Results of a 12-week Pilot Programme

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Aim: To document preliminary results of a 12-week group program in fibromyalgia patients.

Methods: An interdisciplinary team consisting of physician, physical therapists, occupational therapists, nurses, dieticians and psychologists conducted the 8-session, 3-hourly program over 12 weeks. Interventions

include education, stretching and aerobic exercises, proper body mechanics, stress and pain management and dietary advice.

Results: Four patients participated in the program. Six-minute walk test distance improved from a mean of 345.2 ± 57.3 metres to 422.8 ± 60.6 metres post program. Knowledge of good dietary practice improved (9.0 ± 1.4 vs. 14.3 ± 1.5). Beck depression scale (17.2 ± 8.3 to 14.5 ± 17.9) and number of moderate to severe tender points (14.3 ± 1.4 to 17.1 ± 4.8) did not improve significantly. There was no difference in BPI pain scores (4.4 ± 1.8 vs. 4.1 ± 1.9 post) or SF36 scores (physical scores pre- and post-programme: 32.8 ± 8.7 and 34.1 ± 10.0 ; mental scores: 51.2 ± 12.5 and 46.8 ± 13.2 respectively). Fibromyalgia Impact Questionnaire VAS scores for pain, sleep, morning stiffness, anxiety and depression did not improve (33.2 ± 16.5 and 34.5 ± 10.8 respectively).

Conclusion: Physical, cognitive-behavioural and educational group programme for fibromyalgia patients improved 6-minute walk test scores. There was no significant change in pain perception and quality of life. A larger study is required to confirm these results.

II065/MPC

Effectiveness of a Dedicated Non-invasive Ventilation Unit in a General Hospital

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Aim: Non-invasive ventilation (NIV) is a recognised modality in the management of patients with acute respiratory failure (ARF). It can be performed in the general ward setting. We evaluated the outcomes of episodes of ARF treated by NIV in a dedicated intermediate care unit, located in the general medicine ward.

Methods: We conducted a retrospective review of the data of patients who were admitted into the NIVU (Non-invasive Ventilation Unit) from November 2001 to April 2003. General ward nurses, with no previous ICU experience, received training to staff NIVU and were supported by respiratory therapists. A total of 121 episodes (mean age 71.4 years, M:F 2.6:1) of ARF were submitted to NIV in NIVU. We analysed pH and pCO₂ prior to NIV and 2 hours NIV, and also the success rate of NIV, which was defined as resolution of ARF without need for endotracheal intubation or leading to death.

Results: Commonest causes of ARF were decompensated COPD (67.5%), obesity-hypoventilation syndrome (6.7%), bronchiectasis (5.8%) and pneumonia (9.2%). Rate of NIV success was 82.9%. Overall, mean pH and PaCO₂ of all cases improved 2 hours after initiation of NIV [7.27 versus 7.32 ($P < 0.001$) and 77.6 mmHg versus 68.5 mmHg ($P < 0.001$) respectively]. Mean duration of NIV was 4.7 ± 6.1 days. One patient was intubated (twice) but died eventually. Nineteen patients died, majority of whom had end stage COPD and no further intervention was planned.

Conclusion: NIV is effective and feasible in treatment of ARF in a dedicated intermediate care unit.

II066/MPC

Clinical and Radiologic Features of Patients with Mycobacterium Avium Lung Disease

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Aim: Non-tuberculous mycobacteria (NTM) lung disease is an emerging infection with little published clinical data. *Mycobacterium avium* complex (MAC) is relatively common in settings with AIDS. However, MAC lung disease is a rare entity and tends to occur among patients. This study assesses the clinical and radiologic features in an area with a high incidence of TB which may confound its diagnosis.

Methods: All cases with accessible records on follow up at 2 clinics with an interest in NTM lung diseases were reviewed. Cases with lung disease due to *M. avium* complex were analysed. Data regarding demography, clinical and radiologic presentation were analysed.

Results: Fourteen patients were studied (age 63.7 ± 11.9 years, 7 males and 7 females). Cough was the main presenting symptom in 11 patients while hemoptysis was in 4. Two subjects were diabetic. Only 4 were smokers and 3 consumed alcohol. None had HIV. A history of previous TB was noted in

8 subjects, of which 3 had positive cultures recorded. Multiple cultures were obtained from these patients (3.6 ± 1.9 , min. 1, max. 8). Chest x-rays at presentation showed bilateral involvement in 8 subjects. In all 14 cases, upper lobe involvement was noted. HRCT was done for 10 subjects. Most had multi-lobe scarring or disease with only 1 subject having unilobar disease (3.6 ± 1.4 lobes). Treatment was initiated in 11 patients.

Conclusion: MAC lung disease has a similar presentation as pulmonary TB. Lung involvement tends to be extensive and the condition also does not appear to be related to any immune suppression. Clinical differentiation between the 2 illnesses is thus made difficult.

II067/MPC

Osteoporosis is an Unfamiliar Illness Among Elderly Singaporean Patients with Fragility Fractures

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Aim: The HSDP programme for osteoporosis treatment aimed to treat patients who have prior history of fragility fractures to prevent recurrent fractures. This paper examined the reasons why patients refused to join the programme.

Methods: A total of 410 patients were screened using the DRG for hip or spine fractures (2001 to 2002). Face to face or telephonic interviews were conducted and questions on patient's current mobility status, their understanding of osteoporosis and its treatment, and their reasons for not participating in the programme were assessed.

Results: Out of the 410 patients screened (49 spinal fractures and 362 hip fractures), 62 patients had passed away when the screening was done. Only 16% were told that they had osteoporosis after their fragility fracture and only 8% were on anti-resorptive treatment. Of the 221 patients who fulfilled the entry criteria, only 18 were keen to join the programme. Older age (OR 0.93, CI 0.88 to 0.99) and lack of awareness of diagnosis of osteoporosis (OR 0.06, CI 0.02 to 0.22) were associated with lower odds of joining the programme. The common reasons for rejecting the programme were that "osteoporosis was not important", "medication was too expensive" and "no one to bring to doctor".

Conclusion: Majority of patients who already had either spine or hip fragility fractures did not know that they had osteoporosis. Up to one-third of the patients refused treatment because they thought that the treatment of osteoporosis was not important.

II068/MPC

Comparison of Clinical Features of Patients with High Titre Anti Nuclear Antibodies (ANA) in Singapore and Sydney

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Aim: Patients with Connective Tissue Diseases (CTD) of Asian origin appear to have more severe end-organ damage than Caucasian patients. To investigate this, patients of a rheumatologist, who had been entered in his Sydney CTD patient database, were compared to his CTD patients seen at the Singapore General Hospital. The referral pattern was similar in the 2 populations.

Methods: Only patients with a high ANA reading were included in the data analysis. The patients' age, gender, racial origin, diagnosis, use of prednisolone, immunosuppressive therapy and organ involvement were collected prospectively by a clinician. Diagnosis satisfied the relevant ACR criteria for a specific CTD.

Results: The mean age and gender of the 2 populations were similar. Only 10 patients in Sydney satisfied the clinical diagnosis of SLE (25.6%) compared with 20 Singaporean patients (57.1%) ($P = 0.005$). The use of prednisolone was higher in Singapore ($P < 0.0001$). Musculoskeletal features were significantly more common in the Sydney population with 76.9% of patients, compared to only 20% of Singaporean patients. The number of patients on DMARDs was similar, with mainly hydroxychloroquine (21 patients) in Sydney, while 12 Singaporean patients were on strong immunosuppressives like azathioprine and cyclophosphamide.

Conclusion: The differences in CTD manifestations between the Caucasian races and the Asian populations have not previously been so clearly identified. These differences account for the drug use and complications of disease. This needs to be considered in allocation of funds for treatment, research and patient education.

II069/MPC

Erythrocyte Sedimentation Rate Reflects Disease Activity but is not Associated with Damage Accrual in Systemic Lupus Erythematosus

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Aim: An elevated erythrocyte sedimentation rate (ESR) is common in active systemic lupus erythematosus (SLE) and is used to monitor disease course, even though its value is not yet clear. We sought to investigate if ESR was associated with disease activity, damage accrual and quality of life in our SLE patient cohort.

Methods: SLE patients (fulfilling ACR criteria) were consecutively enrolled into a prospective study cohort. Demographic, clinical and laboratory data, SLEDAI and SLAM disease activity indices, the SLICC damage index, quality of life index (SF-36) and the Rheumatology Attitudes Index (RAI) were collected. ESR was divided into 4 categories: <25 (normal), 25-50 (mild), 51-75 (moderate) and >75 (severe).

Results: The cohort mainly consisted of Chinese (78.9%) females (90.9%). At recruitment, the median SLEDAI score was 2 (range, 0-39) median SLAM score 2 (range, 0-28), median SLICC of 1 (range: 0-8) and elevated ESRs were found in 36% of patients. Significant difference between ESR categories were found for disease activity indices but not SLICC or quality of life using Kruskal-Wallis test with Bonferroni's correction.

Conclusion: ESR is commonly raised in SLE, but anti-dsDNA antibody levels do not appear to directly influence ESR. Higher ESRs are associated with more active disease but not with damage accrual or worse quality of life, except maybe in the physical aspects.

II070/MPC

SLAM and SLEDAI are Valid Disease Activity Measures in Oriental Lupus Patients with SLEDAI Being More Sensitive to Change

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Aim: SLAM and SLEDAI, systemic lupus erythematosus (SLE) disease activity assessment instruments, have been developed mainly from studies of Caucasian patients. We sought to compare SLAM and SLEDAI against the physician global assessment of disease activity (PGA) in our cohort of Oriental lupus patients.

Methods: A prospective SLE cohort consists of 460 SLE patients (fulfilling ACR classification criteria for SLE) who were enrolled between May 2002 and December 2003. Assessments were made at fixed intervals with demographic, clinical and laboratory information collected using a standard protocol, disease activity assessed using both SLEDAI and SLAM. PGA was scored using a visual analogue scale (VAS) of 100 mm. The cut-off to differentiate clearly active versus mild/non-active disease was 7 points for SLAM, 4 points for SLEDAI and 30 mm for the PGA.

Results: The cohort comprised mainly Chinese (78.9%) females (90.9%), mean age 41 ± 13 years, median disease duration 7.1 years (range, 0-36). Significant correlations were found in SLEDAI-SLAM, PGA-SLEDAI and PGA-SLAM (ρ : 0.48, 0.33 and 0.50 respectively, $P < 0.0001$ for all) with SLAM having stronger correlation with the PGA. Standardised response means (SRM) and effect size for SLEDAI, SLAM and PGA were used to evaluate their sensitivity to change between 2 consecutive visits and all were shown to have very good sensitivity (0.80).

Conclusion: Using PGA as the gold standard, SLEDAI and SLAM were found to be valid disease activity measures in Oriental patients.

II071/MPC

Outcomes of Chronic Hepatitis B Infection in 23 Oriental Patients Receiving Immunosuppressive Therapy for Rheumatic Disease

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Aim: To describe outcomes of chronic hepatitis B (HBV) infection following immunosuppressive therapy in 23 consecutive oriental patients with rheumatic disease.

Methods: Retrospective case series.

Results: There were 7 (30.4%) males and 16 (69.6%) females, predominantly Chinese (91.3%), with mean age 48.8 ± 15.2 years. Mean duration of rheumatic disease was 9.4 ± 12.4 years, with rheumatoid arthritis (56.5%), systemic vasculitis (17.4%) and systemic lupus erythematosus (8.7%) being the commonest. Chronic HBV infection, mean duration 6.3 ± 7.6 years, was diagnosed during pre-methotrexate screening (43.5%), asymptomatic transaminitis (21.7%), systemic vasculitis (17.4%), and primary care screening (17.4%). At baseline, all patients had normal ALT, 25.0% positive hepatitis B e antigen (HBeAg) and 68.4% positive anti-HBe antibody. Following immunosuppressive therapy, ALT remained normal in 8 patients. Fifteen (65.2%) developed ALT elevation, which was $>2\times$ normal in 9 patients. These patients received prednisolone (2); prednisolone with azathioprine (2), cyclophosphamide (2), hydroxychloroquine (2), sulphasalazine (3) or hydroxychloroquine/sulphasalazine (2); or hydroxychloroquine (2) or sulphasalazine (1) respectively over 10.9 ± 14.6 years. ALT normalised spontaneously in 9 patients without hepatic decompensation or change in therapy. 4 (26.7%) patients, of whom 3 had HBeAg-negative viremia and 1 cirrhosis diagnosed histologically, received lamivudine over 18.8 ± 10.1 months. There were 2 HBV-unrelated deaths from pyogenic infection. None developed hepatocellular carcinoma.

Conclusion: Elevated ALT occurred in 65.2% of rheumatological patients with chronic HBV infection following immunosuppressive therapy, although only 26.7% required lamivudine. There was no HBV-related mortality.

II072/MPC

Lupus Myocarditis Presentation and Outcome

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Aim: Symptomatic myocarditis in SLE is uncommon. Our aim is to study the clinical characteristics, management and outcome of SLE patients with acute lupus myocarditis.

Methods: Retrospective review of 11 SLE patients with a history of acute myocarditis between 1993 and 2001.

Results: All 11 patients were female, the majority being Chinese (46%). Mean age at diagnosis of SLE was 27 ± 10 years. Acute myocarditis was the first presentation of SLE in 8 patients (73%). The most common symptoms and signs were dyspnea (91%), fever (55%), orthopnea (45%), basal crackles (73%), tachycardia (55%), raised jugular venous pressure (55%) and gallop rhythm (45%). Median SLEDAI during acute myocarditis was 16. Laboratory investigations showed raised ds-DNA (100%), low C3 (73%), low albumin (64%), lymphopenia (64%), raised creatinine (36%) and raised creatinine kinase (27%). CXR abnormalities were cardiomegaly (100%), pulmonary congestion (73%) and pleural effusion (73%). Non specific ST/T changes on ECG were common (91%). Echocardiographic changes were WMA (81%), low LVEF (81%), pericardial effusion (73%) and pulmonary hypertension (64%). All patients received high dose of oral corticosteroids, IV methylprednisolone 45% and IV cyclophosphamide 64%. There were 2 deaths (18%) but no recurrence of myocarditis on follow-up for a median duration of 4 years. All survivors had improvement of cardiac function. The majority of the 9 survivors (67%) did not suffer any permanent damage (SLICC score of 0).

Conclusion: Majority of our patients had myocarditis at initial presentation of SLE. Early treatment with high dose corticosteroids and IV cyclophosphamide resulted in good outcome.

II073/MPC

A Comparison Between Foreign Workers from the Indian Subcontinent and Ethnic Indians Living in Singapore: Looking at How a Difference in Lifestyle Affects Obesity, Blood Pressure and Serum LipidsB LIM¹, N OTHMAN², SC LOKE³, L LEONG⁴¹Diabetes and Endocrine Centre, Tan Tock Seng Hospital, Singapore, ²Department of Nursing, Tan Tock Seng Hospital, Singapore, ³Department of Medicine, Tan Tock Seng Hospital, Singapore, ⁴Health Enrichment Centre, Tan Tock Seng Hospital, Singapore

Aim: 111,000 migrant workers of ethnic Indian origin work in Singapore, mainly in blue-collar jobs with an average monthly wage of S\$900. In contrast, the average income of resident ethnic Indians is about S\$4,500. The disparity in disposable income between the 2 groups is associated with a marked difference in lifestyle. Two community surveys focusing on body mass index (BMI), mean arterial blood pressure (MAP), and total cholesterol (TC) were done, comparing these 2 populations. The objective of this study is to compare basic anthropomorphic and metabolic data between migrant workers and residents of ethnic Indian origin. This will enable us to determine if the disparity in lifestyle is enough to cause a significant difference in the measured parameters.

Methods: The data from the 2 groups is evaluated with a 2-tailed *t*-test with unequal variances, giving *P* values and hence confidence limits for the respective data sets.

Results: Unpaired *t*-test results of migrant workers vs. residents are as follows: BMI difference -1.55, *P* value 0.0028 (95% CI -2.56 to -0.54), MAP difference +2.25, *P* value 0.0400 (95% CI 0.11 to 4.59), TC difference -0.65, *P* value <0.0001 (95% CI -0.86 to -0.44).

Conclusion: The difference in lifestyle between migrant workers and residents of ethnic Indian origin in Singapore results in a significantly lower BMI and TC, but higher MAP in the migrant workers.

II074/MPC

Measuring Improvement in Stroke Management in NHG InstitutionsBH HENG¹, N VENKETASUBRAMANIAN², TS CHEAH³, A YIN¹, JM HENG¹¹Disease Management, National Healthcare Group HQ, Singapore,²Department of Neurology, National Neuroscience Institute, Singapore,³Administration, National Healthcare Group HQ, Singapore

Aim: The National Healthcare Group (NHG) carried out audits on stroke management in 2002 and 2003 to drive quality improvement and evaluation of stroke care in its 4 institutions.

Methods: A random sample of 105 cases from each institution was drawn from patients discharged in 2002 with a primary diagnosis of stroke (ICD 430-436). Medical records were reviewed to assess the various clinical domains. Data was analysed using SPSS, and a probability of <0.05 considered statistically significant.

Results: Of the 420 cases selected, a total of 373 cases (88.8%) were audited. The 2003 sample was similar and comparable with that of 2002, and representative of the stroke population in NHG. Brain scanning within 24 hours of admission was carried out in 85.8% of cases (75.6% in 2002) (*P* = 0.001). Blood glucose testing on admission was achieved in 97.3% of the cases. ECG within 24 hours of admission was achieved in 92.8% of cases. Both swallowing assessment and use of care paths were comparable with that of 2002, being 67.6% and 64.7% respectively. Discharge planning was documented in 97.7% of stroke patients who were ADL-dependent. Anti-platelet therapy was instituted in 88.5% of patients with ischaemic stroke (74.1% in 2002) (*P* <0.0001). Communication of prognosis was documented in 83.4% of cases; and that of risk education to patients and relatives was 46.6% (22.4% in 2002) (*P* <0.0001).

Conclusion: The 2003 audit has shown significant improvements in stroke care. There were however, areas of lapses that need to be addressed.

II075/MPC

The Impact of a Multi-dimensional Health Promotion Programme for Older Persons on Physical Fitness and Performance MeasuresCH WONG¹, SF WONG², MY AZIZAH³, YJ WU³, WS PANG²¹Geriatric Unit, Singapore General Hospital, Singapore, ²Department of Geriatric Medicine, Alexandra Hospital, Singapore, ³Clinical Trials and Epidemiology Research Unit, Singapore

Aim: We examined the impact of a multi-dimensional health promotion programme, Health for Older Persons (HOP), on the physical fitness and performance measures of older participants. HOP was designed using Rowe and Kahn's model for successful aging.

Methods: In a quasi-experimental study, 117 community-dwelling, ambulant, nondisabled participants >50 years old were recruited. The main outcome measures were weight, body mass index (BMI), bioelectrical impedance for body fat composition, gait velocity, sit-to-stand time and grip strength. Physical fitness and performance were measured at 0, 6 and 12 months. Repeated measurement analysis was performed for the outcomes. The difference among baseline, 6 and 12 months was examined adjusting for basic demographic, health and social contact factors.

Results: The mean age of participants was 62.9 ± 8.0 years. The response rate was 70.1% at 6 months (*n* = 82) and 64.1% at 12 months (*n* = 75). There was a significant decrease in weight, BMI and bioelectrical impedance between baseline and 12 months. There was an increase in gait velocity; 0.15 m/s (95% CI 0.08 to 0.22, *P* <0.001) at 6 months and an increase of 0.20 m/s (95% CI 0.14 to 0.27, *P* <0.0001) at 12 months from baseline. A decrease in sit to stand time of 0.20s (95% CI -0.08 to -0.31, *P* = 0.0014) and 0.36s (95% CI -0.11 to -0.61, *P* = 0.0070) respectively. There was no change in grip strength.

Conclusion: A multi-dimensional approach to health promotion in older adults, modelled after the concept of successful aging, conferred benefit to participants in terms of improvement in their physical fitness and performance.

II076/MPC

Preventing Readmissions of Stable Heart Failure Patients by Effective Management at Primary Care Through Second Tier Specialised ClinicsYJ LEW¹, S EMMANUEL², PS GOH³¹Medical, National Healthcare Group Polyclinics, Saint Kitts and Nevis,²Medical, National Healthcare Group Polyclinics, Singapore, ³Nursing/

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Aim: To demonstrate that readmissions can be prevented in stable heart failure patients by effective management of heart failure through second tier specialised clinics in a primary care setting, using disease management pathways and managed by a healthcare team led by a family physician.

Methods: A project was designed and implemented whereby stable heart failure patients were discharged to the NHG polyclinics for follow up. These patients are then seen in the polyclinics' second tier clinic, which is run by a multidisciplinary team. The project is funded by MOH's HSDP.

Results: Between February 2004 and April 2004, 23 patients were discharged for follow up in 6 NHG Polyclinics. The majority of patients were males (91.3%), Chinese (78.3%), and were aged between 50 to 79 years (82.6%). Five patients had diabetes mellitus (21.7%), 21 had hypertension (91.3%) and 14 had lipid disorders (60.9%). 80% of the patients had HbA1c of less than 10%, while 52.1% had blood pressures of less than 140/90 mmHg. The large majority (91%) of them belonged to the NYHA Class I. Almost all (95.7%) of the patients were on both beta-blockers and ACEI/ARB, which are the current recommended heart failure medications.

Conclusion: This is the first study undertaken in which stabilised patients with heart failure are seamlessly discharged for follow up in second tier specialised clinics in a primary care setting. The majority of the patients have co-morbid conditions as well.

II077/MPC

Spirometry in Primary Healthcare: A Utilisation SurveyPN CHONG¹, WF CHONG², KC ONG³¹Medical, National Healthcare Group Polyclinics, Singapore, ²CPMP-

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Aim: Although spirometry is useful for the diagnosis and management of patients with airflow abnormalities such as bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD), primary care physicians

rarely use spirometry in routine practice. We reviewed the utilisation and standards of spirometry since the implementation of this diagnostic service in the National Healthcare Group (NHG) polyclinics.

Methods: Newly trained technicians, who underwent regular training sessions, performed the tests using standard portable spirometers. The utilisation rates and quality of the test results over a 6-month period (November 2003 to April 2004) were reviewed. Decision on the acceptability and interpretation of the tests were made according to established standards for performance and interpretation of spirometry.

Results: A total of 1637 tests were performed. 21% of the tests were performed for COPD management, 29.4% for asthma management, 37.8% for diagnosis of asthma and 11.8% for diagnosis of COPD in symptomatic smokers. 31.3% of the tests showed obstructive abnormality, 13.6% suggested a restrictive pattern, 27.6% were normal, and 27.5% were unacceptable for interpretation. The indication and the results of spirometry were differentially correlated ($P < 0.001$). The percentage of unacceptable tests per month has been gradually decreasing from 37.9% in the first month to 18.6% in the final month.

Conclusion: Provision of spirometry in primary healthcare has led to substantial utilisation with defined indications. The majority of tests performed were of acceptable standard and diagnostic value, and the quality of test results improved with technicians' experience and training.

II078/MPC

Early Detection and Intervention of Chronic Obstructive Pulmonary Disease in Smokers in Primary Healthcare

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Aim: With the availability of spirometry service in primary healthcare, early detection and intervention of chronic obstructive pulmonary disease (COPD) in smokers to prevent further deterioration of their lung function are now possible in the community.

Methods: Patients who were more than 40 years old, with a history of smoking but no prior history of COPD or bronchial asthma and presenting with chronic cough and/or breathlessness, were sent for spirometry in the 9 National Healthcare Group Polyclinics from November 2003 to April 2004. These patients had their lung function test results interpreted by their doctors and were managed according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.

Results: A total of 192 patients had spirometry for diagnosis of COPD. The mean age was 57.4 (± 14.4) years and 86.5% were males. 22.9% of the tests showed obstructive abnormality, 15.1% suggested a restrictive pattern, 35.4% were normal and 26.6% were unacceptable for interpretation. Amongst those with COPD, 34.1% were mild, 50.0% were moderate, 13.6% were severe and 2.3% were very severe.

Conclusion: The availability of spirometry in primary healthcare has made the early detection of COPD possible. This will facilitate early and important intervention, which will have maximal influence on the patients, such as smoking cessation and appropriate treatment, to prevent further disease progression and complications in these patients.

II079/MPC

Smokers with Acute Upper Respiratory Tract Infection (URTI): A Study of Their Symptoms and Outcomes

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Aim: Comparison of symptoms and outcomes between smokers and non-smokers who attend for URTI.

Methods: In a prospective study, 595 consecutive patients who attended for URTI were subjected to a questionnaire covering possible symptoms related to URTI. Patients were followed up till resolution of their symptoms. Outcomes captured were antibiotics prescribed, sick leave given, total number of days sick, facial congestion, ear pain and number of cigarettes smoked.

Results: Smokers reported 0.60 times (CI 0.4, 0.9) less itch in the throat than non-smokers. They were also 2.3 times (CI 1.5, 3.6) more likely to report weakness. However, there were no significant differences in the reporting of cough, runny nose, sore throat, fever or shortness of breath. There was no significant difference in the antibiotics prescribed between groups, the total number of days sick nor the incidence of facial and ear pain. Sick leave was found to be given 3.7 times (CI 2.4, 5.7) more in smokers. 70% (83) of current smokers reported a decrease in the number of cigarettes smoked during the episode of URTI, while 4.2% (5) stopped smoking altogether.

Conclusion: Smokers experienced less localised but more constitutional symptoms. Smoking status did not confer an increase risk of prolonged symptoms or complications. However, many smokers do actually cut down the number of sticks smoked during a URTI. This makes it a golden opportunity for the doctor to promote smoking cessation to these patients during the consultation for URTI.

II080/MPC

Polymorphisms of the Insertion ACE Gene and M235T AGT Gene and Essential Hypertension

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Aim: Renin-angiotensin system gene polymorphisms are explored as genetic determinants of essential hypertension. This study investigates whether polymorphisms of the insertion/deletion ACE gene and the M235T AGT gene account for prevalence of hypertension.

Methods: Rapid fragment length polymorphism (RFLP) and restriction analysis determined the frequencies of gene polymorphisms in 637 hypertensive patients and 720 samples from normotensive local blood donors in Saxony, Germany.

Results: No differences were observed in ACE allele and genotype frequency distribution between 2 groups with respect to gender and age. ACE isoforms were of identical frequency within both groups, while AGT TT homozygotes were more frequent in controls (4.6% vs 2.7%, $P = 0.08$). In females, this finding became significant ($P = 0.035$), but not in males. AGT TT genotype was associated with a 48% decrease in the odds of having hypertension (OR-TT vs MM: 0.52; 95% CI: 0.28 to 0.96, $P = 0.034$), and the odds decreased more significantly in women (OR: 0.28; 95% CI: 0.1 to 0.78, $P = 0.01$).

Conclusion: This study does not support that the ACE I/D polymorphism contributes to essential hypertension. In contrast to published data, the M235T TT genotype of AGT gene was detected to confer significantly decreased odds for the development of hypertension in this particular population.

II081/MPC

Development of a Computational Platform to Evaluate Effect of Bone Adaptation on Fracture Susceptibility

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Aim: Osteoporosis is a worldwide public healthcare problem. The number of hip fractures due to osteoporosis exceeded 1300 in Singapore in 1998. Many variants of bone adaptation methods have been proposed; however, a common problem confronting these methods is that the bone density or strain energy remains inconsistent at the nodes and element boundaries, which leads to computing errors and affects numerical stability. Here, a computational platform was developed to evaluate the effect of bone functional adaptation on bone fracture susceptibility.

Methods: A bone-volume based non-continuum formulation is presented to evaluate the effect of bone adaptation on bone properties. The new formulation shifts traditional state variables of adaptation from density to volume, introduces the connectivity matrix and is computationally less demanding. It starts from a uniform distribution of material and progresses toward the adapted structure. The bone fracture susceptibility is then estimated through the effective stress range.

Results: With the hypothetically uniform distribution, bone fracture susceptibility under daily activities is typically greater than 95%, but as the adaptive process goes on, the effective stress range gradually decreases, and

the fracture susceptibility drops below 5% in the final stage, indicating well-adapted trabecular structure.

Conclusion: The bone-volume based non-continuum formulation is a novel method for modelling the bone adaptation process that eliminates the problems commonly encountered before and is computationally less demanding. Linking it to clinical data may allow it to predict fracture risk.

II082/MPC

How Much do Diabetics Know About Diabetes Mellitus and its Complications?

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Aim: Two Singapore studies reported that 99% of diabetics had received some diabetes mellitus (DM) education and the public is generally well informed about DM. Our Phase 1 study indicated that the knowledge level possessed by diabetics and non-diabetics (NDM) was comparable and that a knowledge-practice disparity exists amongst diabetics. The authors aimed to determine the DM knowledge level possessed by diabetics and NDM by visiting 2 NHG polyclinics located in HDB heartlands and the knowledge-practice gap amongst diabetics in this group.

Methods: A face-to-face survey using a questionnaire was conducted. Respondents answered 43 questions, divided into 5 sections: general knowledge, risk factors, symptoms and complications, treatment and management, and monitoring. One point was awarded for each correct response, and 0 for incorrect and unsure responses.

Results: 509 subjects were interviewed. Mean score obtained (maximum: 43) by diabetics and NDM were 32.0 and 29.9, respectively. Mean score obtained by diabetics and NDM for each section respectively: 4.2 and 3.8 upon 8 (General Knowledge); 3.8 and 3.9 upon 6 (Risk Factors); 9.9 and 9.0 upon 12 (Symptoms and Complications); 10.8 and 10.1 upon 13 (Treatment and Management); 3.2 and 3.2 upon 4 (Monitoring).

Conclusion: Unlike the phase 1 study, polyclinic diabetics scored higher than NDM in diabetes knowledge. Less than a third of diabetics practised home blood glucose monitoring. The knowledge-practice gap was smaller as suggested by the high percentage of diabetics practicing 80% of DM self-care items, indicating that diabetes education resulted in better informed diabetics and changed practices.

II083/MPC

Tuberculosis Post Liver Transplantation

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Aim: Tuberculosis (TB) is a rare complication after organ transplantation but is sometimes fatal. We report a case of pulmonary TB post-liver transplantation and discuss its presentation and management.

Methods: From 1990 till May 2004, 100 liver transplants had been performed at the NUH Liver Transplantation Programme, of which 1 patient developed pulmonary TB post-transplantation.

Results: A 60-year-old Indonesian male, with no past or contact history of TB, underwent orthotopic liver transplantation for chronic hepatitis C-related decompensated cirrhosis and hepatocellular carcinoma. Pre-transplant chest X-ray and CT scan of thorax were completely normal. Three-and-a-half months post-transplant, the patient presented with fever, acute confusion and rapidly progressive right upper lobe pneumonia. Pulmonary TB was diagnosed on positive sputum smear for acid-fast bacilli and TB culture. Initial anti-TB treatment consisted of 6 months of isoniazid, ethambutol, ofloxacin and rifampicin. Rifampicin was substituted with streptomycin 4 weeks later due to drug-induced cholestasis. Immunosuppression regime was adjusted: prednisolone tapered from 9 mg daily to 0 over a 6-month period, while cyclosporine increased from 125 mg b.d. to 175 mg b.d. No acute rejection was observed. Patient was last reviewed 28 months post TB-treatment and remained well.

Conclusion: Pulmonary TB could present atypically as rapidly progressive pneumonia in organ transplant recipients, and should be suspected even

without past or contact history of TB, or radiological evidence of prior pulmonary TB on pre-transplant chest X-ray, especially in patients from endemic areas. High level of suspicion, prompt anti-TB treatment, awareness of drug-induced liver toxicity, and close follow-up are essential in management.

II084/MPC

States of Severely Altered Consciousness Clinical Characteristics Medical Complications and Functional Outcome After Acute Rehabilitation

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Aim: To document injury variables, medical complications and functional outcome in patients presenting in states of severely altered consciousness (SSAC).

Methods: A retrospective case series of 30 consecutive patients in persistent vegetative states (PVS) or minimally responsive states (MCS) rehabilitated in an inpatient brain injury rehabilitation unit. Functional outcome measures included admission and discharge Disability Rating Scale (DRS), Ranchos Los Amigos Scale (RLAS) and Modified Barthel Index (MBI).

Results: Altogether, there were 17 (57%) males and 13 (43%) females (mean age 31.8 years, SD 16.3 years, range 15-74 years). Twenty-one (70%) had traumatic brain injury. Seventeen (57%) patients were in PVS and the rest were in MCS on admission to rehabilitation (RLAS levels II and III). Mean acute and rehabilitation length of stays (LOS) were 90.1 (SD 50.3) and 106.3 (SD 39.1) days respectively. Tracheostomised patients had a longer acute LOS ($P = 0.03$). Twelve patients progressed to a state of awareness and a greater spread of higher RLAS categories on discharge was seen. Urinary tract infection (UTI) in 16 was the commonest medical complication. All patients demonstrated positive gains in DRS scores upon discharge from rehabilitation. The MBI was generally insensitive to functional change post-rehabilitation, although paired analyses were significant. The majority of patients (80%) were discharged home.

Conclusion: The extent of initial disability predicted a worse functional outcome in this cohort. While the majority showed cognitive improvement after inpatient rehabilitation, profound disability persisted.

II085/MPC

Psychological Morbidity and Stigma of Severe Acute Respiratory Syndrome (SARS) among Healthcare Workers in Singapore

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Aim: This study was aimed at exploring the psychological morbidity and perception of stigma among healthcare workers following a suspected SARS outbreak at the Institute of Mental Health, Singapore.

Methods: The staff of the Institute were assessed using 3 self-report questionnaires; the GHQ-28 which assessed psychological distress, the Impact of Events Revised Scale, which assessed the symptomatic status with respect to the 3 domains of Post Traumatic Stress Disorder, and the perception of stigma as experienced by the subjects was assessed with a questionnaire adopted from the HIV Stigma Scale. The relevant sociodemographic data was collected using a structured questionnaire.

Results: A total of 443 members (32.6%) of the staff responded to the survey, of which 170 (38.4%) were males and 273 (61.7%) were females. Using a threshold of 5/6 on the GHQ-28, 98 respondents (22.1%) were classified as a "GHQ-case".

Conclusion: We found a high prevalence of psychiatric morbidity among our healthcare workers. Female healthcare workers were significantly more likely to be a case ($P = 0.01$) as compared to male healthcare workers. Doctors scored higher on GHQ sub-scales while nurses scored higher on the stigma subscales. Only 21 members (4.7%) of the staff received psychological counselling through a confidential help line that was set up for all the hospital staff. Steps must be taken to protect healthcare workers against the more covert psychological effects of global epidemics.

II086/MPL**EWS-WT1 Fusion Transcript in the Peritoneal Effusion of a Patient with Desmoplastic Small Round Cell Tumour**L CHIU¹, ESC KOAY², NNH CHAN², M SALTO-TELLEZ²¹Department of Clinical Measurement Unit, National University Hospital, Singapore, ²Department of Pathology and Laboratory, National University of Singapore, Singapore

Aim: Desmoplastic small round cell tumour (DSRCT) is a highly aggressive tumour that often occurs as multiple masses in the abdomen involving the regional lymph nodes and the lining of the abdomen and pelvis. This rare undifferentiated neoplasm predominantly affects males, usually in their second decade of life. Patients with DSRCT have a poor prognosis, even if therapy is instituted promptly, and an overall survival rate of <20%. We present a case of DSRCT in a 17-year-old male with disseminated peritoneal disease and peritoneal effusion in January 2003. The cytology sample showed a malignant small round cell tumor with the classical cytological features of DSRCT, and immunohistochemistry performed on the prepared cell block exhibited an antibody expression profile in keeping with DSRCT.

Methods: DSRCT is associated with a characteristic translocation between chromosome 11 and 22 involving the EWS and WT1 genes, and identification of this fusion gene is used in the diagnosis of this tumour. An RT-PCR designed to detect the chimeric gene was carried out on the aspirated ascitic fluid.

Results: The result showed presence of the t(11;22) (p13;q11 or q12) translocation. The transcript of the reciprocal translocation was later confirmed by direct sequencing.

Conclusion: This case study demonstrates the usefulness of employing molecular techniques to complement cytology and/or cytogenetics assessment in resolving and confirming certain difficult diagnostic conundrums presented by undifferentiated neoplasms.

II087/MPL**Nasopharyngeal Carcinoma Cell Lines: Sensitivity To TRAIL-induced Cell Death**EH LIM¹, MC LIM²¹Department of Haematology-Oncology, National University of Singapore, Singapore, ²Department of Medicine, Faculty of Medicine, National University of Singapore, Singapore

Aim: Nasopharyngeal cancer (NPC) is endemic in the Chinese population. Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a promising candidate for cancer therapy as it is primarily active against a variety of cancer cells with little effect on normal cells. FaDu, an NPC cell line, was previously found to have a homozygous deletion of the death receptor DR4, and resistant to the cytotoxic effects of TRAIL. In this study, we evaluated the cytotoxicity of TRAIL in several NPC cell lines, including CNE1, HK-1, FaDu and HONE1.

Methods: Cells were treated for 8 to 48 hours with varying doses of TRAIL in the presence and absence of cycloheximide (35 µM). Cell viability was assessed using the WST colorimetric assay. Human fibroblast cell lines (GM01386 and GM07492) and HepG2 were used as negative and positive controls respectively.

Results: After 8 hours of exposure to 1 ng/µL TRAIL in the presence of cycloheximide, about 70% cell kill was achieved in HepG2 (+ve control) but none in the fibroblast cell lines (-ve controls). CNE1 and FaDu were the most resistant (~30% cell kill), with HK-1 exhibiting intermediate cell kill (~50%). HONE1 was the most susceptible to the cytotoxic effect of TRAIL (~80% cell kill).

Conclusion: Our study has demonstrated that there are varying degrees of sensitivity towards TRAIL amongst the different NPC cell lines. The mechanisms underlying the different responses to TRAIL amongst these NPC cell lines are being examined. TRAIL may prove to be a helpful adjunct in current anti-cancer therapeutic regimens for NPC, which involves conventional chemotherapy and radiotherapy, and fraught with toxic side effects.

II088/MPL**Invasive Group B Streptococcal Infections in Non-pregnant Adults**P KRISHNAN¹, WL YEO², MS WONG¹¹Department of Laboratory Medicine, Alexandra Hospital, Singapore,²Department of Medicine, Alexandra Hospital, Singapore

Aim: Beta-haemolytic streptococci with Lancefield's group B antigen (*Streptococcus agalactiae*) are known colonisers of the female genital tract (and the adult gut) leading to colonisation of infants with consequent risk of serious neonatal disease characterised by sepsis and meningitis. Over the last decade however, the spectrum of disease produced by this organism has been changing worldwide, with disease manifestations in non-pregnant adults. In the majority of reported cases, predisposing conditions were identified and these included trauma, immunocompromised state and diabetes.

Methods: A retrospective study was carried out over a 6-month period starting January 2004 to review the spectrum of disease caused by *S. agalactiae* in this 400-bed general hospital (which does not have maternal or neonatal specialties). The laboratory records of all patients with *S. agalactiae* isolated from clinical samples were reviewed.

Results: A total of 15 cases were identified. All isolates were susceptible to penicillin. The spectrum of disease included urosepsis, abscesses (including 2 cases of thyroid abscess), tenosynovitis and bacteraemia. Risk factors included diabetes, geriatric age group, chronic renal disease and underlying malignancy.

Conclusion: It has been postulated that certain capsular serotypes and clonotypes account for the majority of disease in non-pregnant adults. Further work needs to be done in this field to ascertain if this is true in this community as well. A prospective study will also help determine if there is any temporal increase in *S. agalactiae* infections in non-pregnant adults in this hospital.

II089/MPL**The Role of Two Polymorphisms in the Endothelial Nitric Oxide Synthase Gene in Nephropathy Among Singaporean Chinese Patients with Type 2 Diabetes Mellitus**FYA KOH¹, T GOH², XH XU³, SC LIM¹, CF SUM¹¹Department of Medicine, Alexandra Hospital, Singapore, ²Diabetes Centre, Alexandra Hospital, Singapore, ³Clinical Research Unit, Alexandra Hospital, Singapore

Aim: Several allelic variations of the endothelial nitric oxide synthase (eNOS) gene have been evaluated for possible links to cardiovascular disease, renal disease and diabetes mellitus. We studied the role of 2 polymorphisms in the promoter region of the eNOS gene, the T-786C polymorphism and the T-1468A polymorphism, in conferring susceptibility to nephropathy among Singaporean Chinese with type 2 diabetes mellitus (T2DM).

Methods: We compared 2 groups of Chinese patients with T2DM: cases had proteinuria >0.5 g/day or urine albumin/creatinine ratio of >0.5 mg/mg or persistently elevated serum creatinine while controls had T2DM for at least 10 years with consistently normal serum creatinine and urine albumin/creatinine ratio of <0.05 mg/mg. Genotyping was carried out using standard polymerase chain reaction, restriction fragment length polymorphism and electrophoretic resolution.

Results: Fifty cases and 50 controls were genotyped for the T-786C polymorphism, while 46 cases and 48 controls were genotyped for the T-1468A polymorphism. Genotype distribution for both cases and controls conformed to Hardy-Weinburg equilibrium for both polymorphisms studied. For the T-786C polymorphism, the frequency of the C allele was 12% for both cases and controls. For the T-1468A polymorphism, the frequency of the A allele was 9.9% in cases and 8.3% in controls ($\chi^2 = 0.01$, $P = 0.9203$).

Conclusion: We conclude that the 2 polymorphisms studied are not likely to be related to diabetic nephropathy.

II090/MPL**Identification and Characterisation of Deleted in Esophageal Cancer 1 in CD4+ T Cells in Children with Minimal Change Nephrotic Syndrome**CL WEI¹, W CHEUNG¹, CGL LEE², JH LU³, SC JORDAN⁴, HK YAP¹¹Department of Paediatrics, National University of Singapore, Singapore,

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Aim: It has been suggested that minimal change nephrotic syndrome (MCNS) results from a primary immune disturbance with a Th2 cytokine bias. We have demonstrated that interleukin-13 (IL-13) gene expression was upregulated in T cells in MCNS children in relapse. However, its upstream or downstream molecular events are still unclear. This study further investigated the differentially expressed genes in T cells of MCNS, with their functions being characterised.

Methods: CD4⁺ T cells were purified from peripheral blood mononuclear cells (PBMC). Total RNA was subjected to differential display RT-PCR (DDRT-PCR). Differentially expressed PCR products were then cloned and sequenced. The upregulated gene was verified by Northern blotting and RT-PCR, and subsequently transfected into Jurkat cells, followed by proliferation and apoptosis assays.

Results: In addition to IL-13, the deleted in esophageal cancer 1 (DEC1) gene was shown to be significantly upregulated in CD4⁺ but not CD8⁺ T cells during relapses of MCNS. When transfected into Jurkat cells, DEC1 inhibited cell proliferation by $47.2 \pm 4.3\%$. Active caspase-3 staining showed that apoptosis may be responsible at least in part.

Conclusion: DEC1, a potent tumour suppressor gene, was identified in CD4⁺ T cells of MCNS and its expression was significantly upregulated in relapse. As it inhibits cell proliferation and induces apoptosis, it may be important in mediating the functions of T cells in MCNS. Further study is warranted to address its potential relationship to IL-13 during nephrotic relapses.

II091/MPL

Tissue Microarray Analysis of Mammary Phyllodes Tumour

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Aim: Background: Mammary phyllodes tumours (PT) are fibroepithelial neoplasms of the breast. Histologically, PT are characterised as benign, borderline and malignant. The aim of this study was to analyse the expression profiles of a panel of markers in the PT tissue microarray of our study population by immunohistochemistry.

Methods: Seventy-six cases of PT diagnosed from 1991 to 2000 were reviewed, and the area reflecting the characteristics of full sections was selected for tissue microarray construction. Immunostaining for estrogen receptor (ER), progesterone receptor (PR), c-Kit, HER-2/neu, APC and p53 were performed on TMA sections.

Results: ER expression in stroma and epithelium was noted in 1.4% and 71.4% cases, respectively. Similarly, PR expression was 0% and 61%. The concordance of TMA results of ER and PR with the full sections is at moderate level (average kappa value, 0.475). Of the available cases, stromal expression of p53 was found in 67 (93%) cases and epithelial expression of p53 in 56 (91.8%) cases. Twenty-six cases (36%) showed APC expression in stroma and epithelium, respectively. Only 1 case was found to be weakly stained for HER-2/neu in stroma and 32 (44%) cases were positively stained in epithelium. c-Kit expression was observed in 22 (30%) cases whereas only 3 cases showed positivity in stroma. In correlation with histological criteria, only p53 stromal expression showed a significant difference between benign and malignant lesions: 18/41 (44%) benign tumours had p53 expression, whereas 10/10 (100%) malignant tumours revealed p53 expression ($P = 0$).

Conclusion: p53 stromal expression can be a useful marker in predicting malignancy in PT.

II092/MPL

Ocular Melanocytoma: Infrequent but Important Eye Lesion of 2 Cases

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Aim: We reported 2 cases of incidental ocular melanocytoma in 2 young patients. Melanocytomas are well-differentiated neoplasms and must be distinguished from other pigmented lesions of the eye like malignant melanoma, adenoma and adenocarcinomas of the pigmented ciliary epithelium by histopathological criteria.

Methods: Both patients had their affected eyes enucleated and a black tumour was noted in the uvea and choroid. Sections were stained with haematoxylin and eosin. Immunohistochemical stains with S-100, HMB-45 and Ki-67 were used to determine the exact nature of the tumour.

Results: Microscopically, the tumour is well-circumscribed and showed a combination of spindle and epithelioid melanocytes loaded with melanin pigment and obscuring most of the cytoplasmic and nuclear details. The cells showed minimal nuclear atypia and there were no mitoses or necrosis. The tumour cells were positive for HMB-45 and S-100 and Ki-67 was negative. A diagnosis of melanocytoma was rendered.

Conclusion: Melanocytomas can involve the optic disc, choroid, iris and uvea. These lesions are asymptomatic and seldom encountered. It is important for the pathologist to recognise this lesion and not misinterpret it as malignant melanoma. Features indicating the benign nature of this tumour such as minimal nuclear atypia, rare to absent mitoses, low proliferation index and absence of necrosis and invasion should be carefully assessed to arrive at an accurate diagnosis. Since so few of these tumours have been studied, long-term follow-up is needed to accurately assess the biological behaviour of ocular melanocytomas.

II093/MPL

The Performance of a RT-PCR Compared with a Rapid Serological Assay for Acute Dengue Fever in a Diagnostic Laboratory

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Aim: The laboratory diagnosis of dengue has relied on serology although many different RT-PCR protocols have been reported. Due to limited use, the value of RT-PCR in the clinical laboratory has not been fully evaluated. During the outbreak of severe acute respiratory syndrome (SARS) in Singapore last year, we rapidly set up RT-PCR as a tool to differentiate dengue from SARS among the patients that presented to Tan Tock Seng Hospital, the hospital designated to manage and quarantine SARS cases. The objective of this study was to compare the performance of the RT-PCR with a rapid serological assay for the diagnosis of dengue.

Methods: We studied a total of 343 and 439 results for RT-PCR and serology respectively. These were analysed by the day of illness. The clinical discharge diagnosis was the gold standard.

Results: RT-PCR was 100% specific. Its sensitivity declined from 100% in the first 2 days of illness to 76% on day 6 and 52% on day 7 of illness. The serological assay showed a rising sensitivity from 38% on day 6, through 59% on day 7 to reach 80% by day 8.

Conclusion: Our experience indicates that RT-PCR for dengue is a very sensitive and specific tool for the diagnosis of dengue, particularly in the first 5 days from the onset of symptoms, and it can be easily set up in a clinical laboratory. Serology is more useful than PCR after the sixth day of illness.

II094/MPL

Cyclooxygenase and Angiogenesis in Hepatocellular Carcinoma

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Aim: Several cancers are associated with chronic inflammation, and among these is hepatocellular carcinoma (HCC) arising on the background of hepatitis B and C, and cirrhosis. Studies suggest that cyclooxygenases (Cox), which catalyse the conversion of arachidonic acid to prostaglandins are linked to carcinogenesis, particularly the inducible Cox-2. Cox-2 is expressed

highly in well-differentiated HCCs and in low amounts in advanced HCCs. The aim of this study is to investigate the relation between Cox-2 expression and angiogenesis in hepatocellular carcinoma.

Methods: Seventy proven cases of hepatocellular carcinomas from the National University Hospital, Singapore were retrieved and reviewed. Immunohistochemical stains for Cox-2 and CD31 were done. Three pathologists independently graded the Cox-2 expression (product of intensity and range of cells expressing Cox-2). Then, the sections stained with CD31 were evaluated for angiogenesis.

Results: The *P* value of the mean vessel density of Cox-2 negative cases as compared to Cox-2 positive between well-differentiated, moderately differentiated and poorly differentiated HCCs are 0.61, 0.25 and 0.89, respectively. It shows that there is no significant relationship between microvessel density (angiogenesis) and Cox-2 expression on all levels of histologic differentiation of HCCs.

Conclusion: Cox-2 may have no direct influence on angiogenesis in HCCs but may affect other steps of carcinogenesis. Also, studies into other related inflammatory mediators, such as tumour-associated macrophages, as well as expression of other inflammatory chemicals such as tumour necrosis factor (TNF) and interleukins (IL) and their relation to tumour vascularity may reveal the intricate association of inflammation and carcinogenesis.

II095/MPL

Role of Cell Block Immunocytochemistry in the Diagnosis of Lymphomas

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Aim: Traditionally, lymphomas are diagnosed on histoarchitecture, cytomorphology and immunophenotyping. Under the current WHO classification, there is no single gold standard for their diagnosis. Our interest was to examine the extent to which immunocytochemistry on cell blocks of fine needle aspirates (FNAs) could provide diagnostic information.

Methods: We selected 15 cases from the last 2 years with FNAs and subsequent biopsies that were either differentially or finally diagnosed as lymphoma, examining disparities between the FNA and final histological diagnoses, and investigating the extent to which cell block immunocytochemistry could narrow these disparities.

Results: In 12 out of 15 cases, diagnostic information not previously anticipated could be gleaned from their FNA cell blocks by immunocytochemical analysis. In 2 cases, there were insufficient cells for immunostaining, and in one case the cell block had been exhausted.

Conclusion: In the light of this study, we suggest that cell block immunocytochemistry could provide useful diagnostic information in cases of suspected lymphoma where formal excision biopsy might be risky or contraindicated, provided the cell yield is adequate.

II096/MPL

Use of Tissue Microarray (TMA) Technology in Immunohistochemistry: Quality Control and Evaluation of New Antibodies

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Aim: Tissue microarray technology (TMA) is a recent development that facilitates rapid tissue analysis by in situ hybridisation and immunohistochemical techniques. Multiple cores of tissue as small as 0.6 mm and as large as 2 mm in diameter are incorporated into a paraffin block. About 200 tissue sections at 4-mm thickness can be cut from each microarray block.

Methods: Positive control tissues are selected based on the common immunoassays performed in our laboratory. Suitable paraffin blocks containing selected tissues are identified and retrieved from our archives. One or more immunostains are performed using standard tissue sections to ensure the quality of antigenic preservation. Tissue cores of 1-mm diameter are prepared into a TMA block using the Beecher microarrayer. We cut standard 4-mm sections and mount them at one edge of the slide, adjacent to the test material.

Results: For evaluation of new antibodies, we initially use a limited array comprising a variety of tissues including tonsil, colon, thyroid, liver (HBV+),

breast, lung, cerebellar, prostate, testis, placenta, stomach, spleen, adrenal, as well as cancers of the bladder, liver and breast. Depending on the type of tissue showing expression, a more extensive array containing many more examples of the appropriate tissue type is used for optimisation.

Conclusion: We describe a useful method of providing positive control tissue for evaluating staining efficacy in routine immunohistochemistry as well as deciding optimal staining protocols for new immunoassays.

II097/MPL

Detection of c-erb B2 Amplification in Breast Carcinoma: Comparison Between Immunohistochemistry and Chromogen In situ Hybridisation

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Aim: Amplification of the c-erbB2/Her-2 gene or overexpression of its encoded protein is associated with worse prognosis in breast cancers, particularly in node positive cases. Herceptin (trastuzumab), a humanised anti-Her-2 monoclonal antibody directed against the Her-2 protein, has been approved for the treatment of metastatic breast cancer. The demonstration of Her-2 amplification has become an increasingly important prognostic and predictive investigation in breast cancer.

Methods: Thirty cases of breast carcinoma were studied using chromogenic in situ hybridisation (CISH) detection of the c-erb B2 oncogene using published methods. Briefly, a Her-2 specific probe is labelled with digoxigenin, and allowed to hybridise to tissue sections. This is followed by sequential incubations with antidigoxigenin fluorescein, antifluorescein peroxidase and diaminobenzidine. The results are compared with those of immunohistochemistry against the Her-2 protein.

Results: Twenty-six of 30 cases (87%) show complete concordance between immunohistochemistry and CISH. Of the 4 remaining cases, 3 were scored as negative (0 to 1+) by immunostaining but low-level amplification (<10 signals) was noted by CISH. The reverse occurred in one case, in which immunostaining for Her-2 was graded as positive (2+) but CISH clearly showed absence of Her-2 amplification.

Conclusion: There is good concordance between immunohistochemistry and CISH for Her-2 but interpretation of CISH signals is easier and more objective, compared to the semi-quantitative assessment of immunostaining. There is a false-negative rate of 10% and a false-positive rate of 3% for predicting Her-2 amplification when using immunohistochemistry.

II098/MPL

Diffuse Large B-cell Lymphoma with a Late to Post Centre Phenotype: A Study Using Immunohistochemistry and Tissue Microarray (TMA)

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Aim: To study if there is a subset of diffuse large B-cell lymphoma with a late to post-germinal centre cell phenotype that may be determined by immunohistochemistry.

Methods: Thirty cases of diffuse large B-cell lymphoma were selected and the histology was reviewed. We constructed 3 tissue microarray blocks using both the 0.6-mm and 1-mm tissue punches. Immunohistochemistry was performed using CD3, CD20, CD10, bcl6, PAX5, CD138 and MUM-1.

Results: By tissue microarray, 3 cases were not sufficiently representative in the microarray block. The remaining 27 cases featured sufficient tissue cores for immunohistochemical staining. There is a large proportion of highly proliferative tumours with a proliferative fraction of 60% or more (in 19/27 cases). There is a population of diffuse large B-cell lymphoma that expresses MUM1 (7/27), which is a marker of late to post-germinal centre phenotype. However, all but 2 of these cases also co-express Bcl-6, a marker of germinal centre phenotype. What is particularly interesting is that none of these cases co-express CD10, another marker of germinal centre cells, regardless of Bcl-6 expression.

Conclusion: These early findings indicate that there is a subset of DLBCL, which appears to express a late germinal centre to post-germinal cell phenotype. It appears that CD10 rather than Bcl-6 might be a better marker of germinal centre phenotype, since the latter is also co-expressed in cases that express MUM-1.

II099/MPL

Cardioprotective Effect of Des-Aspartate-Angiotensin-I (DAA-I) on Cytokine Gene Expression Profile in Myocardial Infarction
RA JALIL¹, HK HAIDER², MK SIM³, ZP DING⁴, SJ JIANG⁴, L YE¹, EKW SIM¹¹Department of Surgery, National University of Singapore, Singapore, ²National University Medical Institutes, Singapore, ³Department of Pharmacology, National University of Singapore, Singapore, ⁴National Heart Centre, Singapore**Aim:** To investigate the effect of DAA-I on expression profile of inflammatory cytokine genes in rodent model of myocardial infarction.**Methods:** Myocardial infarction models were created in female Wistar rats by coronary artery ligation. They were randomised to receive intravenously, a daily dose of 1.2 µg/kg (body weight) of DAA-I (n = 60) or saline solution (n = 60) for up to 14 days after infarction. Echocardiography was done to assess the heart function. The animals were harvested at 1, 3, 7, 14 and 30 days after infarction (n = 12 at each time point). Infarct size was measured by tetrazolium chloride staining. Immunohistochemical staining was done to assess the extent of immune cell infiltration and expression of inflammatory cytokines at the infarct zone. Multiplex PCR was used to study the differential gene expression of IL-6, IL-1β, TGF-β, TNF-α and GM-CSF between experimental and control groups.**Results:** Significant reduction in infarct size started after day-14 of DAA-I treatment (32.2%, *P* < 0.05). Left ventricular ejection fraction improved significantly after day-3 of treatment. Extensive infiltration of immune cells observed in saline-treated group was reduced in DAA-treated group. IL-6, TNF-α, TGF-β and GM-CSF expression were significantly down-regulated in the infarct, peri-infarct and contralateral zones of the left ventricle in DAA-I-treated group as compared to saline-treated group. Reduction of IL-6, TGF-β and GM-CSF expression started on day-1 after treatment while TNF-α expression only reduced after day 3 of treatment.**Conclusion:** DAA-I reduces infarct expansion through suppression of inflammatory cytokines and immune cells infiltration into the infarct region.

II100/MPL

Oxidative Stress in Diabetes Mellitus: cGMP Levels in Human Umbilical Cord Arteries Incubated in Normal and High Glucose Ambient Concentrations

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Aim: Vascular complications in diabetes mellitus result from increased free radical activity in the presence of high ambient glucose concentration. Such activity has been hypothesised to impair the nitric oxide pathway resulting in endothelial dysfunction. Vascular relaxation mediated by nitric oxide is effected and amplified by its second messenger, cyclic guanosine monophosphate (cGMP).**Methods:** Human umbilical cord arteries were incubated in normal and high glucose conditions and stimulated with nitric oxide. cGMP levels were extracted and measured using radioimmunoassay.**Results:** A trend for lower cGMP levels was observed in arteries incubated in high glucose concentration verses lower glucose concentration. Mean difference 0.639 pmol/g (*P* = 0.28).**Conclusion:** Although this test did not achieve significance due to its small size, the results supported the hypothesis that the nitric oxide pathway is impaired in the presence of high glucose concentration.

II101/NUR

Nurses' Knowledge on Pain ManagementE NASER¹, S SINWAN¹, HB WONG²¹Department of Nursing, Alexandra Hospital, Singapore, ²Clinical Trial Epidemiology Research Unit, Singapore**Aim:** Pain management of a patient is one of the critical responsibilities of a nurse. Despite the availability of the effective analgesics and new technologies in drug administration, studies continue to demonstrate a suboptimal pain management in the hospital. The aim of this study is to determine the pain management knowledge of registered nurses in Alexandra Hospital.**Methods:** Data was collected using validated self administered questionnaires to 237 registered nurses in Alexandra Hospital. Questionnaires used were Nurses' Knowledge and Attitude Survey by Dr BR Ferrel.**Results:** 198 questionnaires were returned. The inpatient nurses' knowledge on pain management was found to be significantly higher (*P* = 0.005). Intensive care unit nurses achieved higher scores. There was no significant difference seen in scores of nurses with longer nursing experiences.**Conclusion:** The results indicate that suboptimal knowledge of pain management exists in Alexandra Hospital registered nurses.

II102/NUR

Quality of Life (QOL) in Young Adults with DiabetesBL HENG¹, MCY TA¹, JST LIM², CL CHUA¹, DYC LOH¹¹Department of Nursing, Alexandra Hospital, Singapore, ²Department of Nursing, KK Women's & Children's Hospital, Singapore**Aim:** Diabetes mellitus is a condition which requires continuous adjustment to lifestyle practices to achieve optimal glycaemic control. Therefore, it can become a burden and interfere with the patient's lifestyle. The purpose of this study was to describe the perception of young adults with diabetes. With an understanding of their perception, the healthcare team can tailor treatment to improve self care management.**Methods:** This study was conducted in a diabetes centre in one of the restructured hospitals. A convenience sample of 25 young adults aged between 18 and 35 years was taken. The subjects were literate in English and had either type 1 or 2 diabetes of 5 years duration or less. A Diabetes Control and Complications Trial (DCCT) Diabetes Quality of Life (DQOL) questionnaire was used as the research instrument. DCCT DQOL questionnaire consists of 4 primary scales of domain: satisfaction, impact, social/vocational worry and diabetes-related worry.**Results:** Our study shows a mean satisfaction level of 67.5% of maximum possible satisfaction score in relation to their treatment and lifestyle. A mean impact of illness score of 41.4% of the maximum possible was noted. The level of social/vocational worries was 45.3% of the maximum possible and diabetes-related worries score was 47.4%. The overall degree of worry affecting the quality of life in our study population was not high.**Conclusion:** In conclusion, the study has shown that diabetes and its management does not appear to interfere significantly with the quality of life of young adult patients.

II103/NUR

Preliminary Experience from a Group-based Diabetes Empowerment ProgrammeT EE¹, MCY TA¹, WKL CHUI¹, MFF CHONG², YH PUA³, KLH NG⁴, PAL ANG³¹Department of Nursing, Alexandra Hospital, Singapore, ²Department of Nutrition & Dietetics, Alexandra Hospital, Singapore, ³Department of Rehabilitation, Alexandra Hospital, Singapore, ⁴Medical Social Work, Alexandra Hospital, Singapore**Aim:** Diabetes self-care education is important in diabetes management. Traditional one-to-one education is effective but is resource-intensive. Therefore, we explored the effectiveness of group-based education in a multi-ethnic population to empower the individual; hence the name of Diabetes Empowerment Programme or DEP.**Methods:** We studied the clinical and metabolic profile of 28 subjects with diabetes at baseline and 6 months after DEP. Among them, 57% were male. 60.7% were Chinese, 28.6% were Malay and 10.7% were Indians. Approximately 90% of them had Type 2. Their mean age was 44 ± 12 years and duration of disease was 10 ± 7 years.**Results:** A significant reduction of mean HbA1c from 9.8 ± 2.7% to 8.2 ± 1.8% was seen over a period of 6 months (*P* < 0.001) after completion of the DEP. There was no significant change in blood pressure. However, there was a slight increase in mean weight from 73.0 ± 15.4 kg to 74.7 ± 17.0 kg (*P* < 0.05).**Conclusion:** It is shown in this study that our 4-week group-based diabetes empowerment programme has a sustained benefit in glycaemic control over a 6-month period. Nonetheless, further studies using a randomised control design with a larger sample size should be considered to examine the effectiveness of group-based education programmes in our population.

II104/NUR**Registered Nurses' Behaviors on Extended Role in Performing Advanced Skills**SZ MORDIFF¹, GN HENG², NK ANG³¹Department of Nursing, National University Hospital, Singapore, ²Health Sciences, previously Nursing, Nanyang Polytechnic, previously NUH staff when research was conducted, Singapore, ³The Cancer Institute, National University Hospital, Singapore

Aim: The role of nurses is evolving towards greater expansion. Training of nurses on the advanced skills to expand their role has been conducted extensively for the past 1 and a half years. However, the impact of the training in changing the behaviour of the registered nurses in performing intravenous cannulation and administering medicines intravenously is inconclusive. This study also aims to explore the barriers that prevent the nurses from undertaking their new role.

Methods: This research is a cross-sectional quantitative survey questionnaire involving registered nurses in the general wards. The study was preceded with a focus group interview of nurses and was piloted to ensure content validity.

Results: A total of 347 (81%) nurses responded. Findings revealed that most nurses are aware of expanded role and benefits. Findings also revealed that most nurses are practising advanced skills. 98% of the respondents agreed that it was important to have sufficient and relevant support to enable them to practise. Areas of concern are litigation issues, devaluing the essential value of "basic" nursing care, perpetuating a "hand maiden" role, reducing nurses' time in providing other nursing activities and increased responsibilities. Interestingly, findings also revealed that there is no reduction in contact time with patients.

Conclusion: Nurses seemed to be performing the advanced roles and are aware of the benefits to patients and themselves when they undertake the expanded role. However, there are still concerns and barriers reported which need to be resolved.

II105/NUR**Survey on Pulmonary Tuberculosis (PTB) Treatment Defaulters**TBYL TAN¹, KY HAN¹, SH GAN², M TELEMAN³, C CHEE⁴, YT WANG⁴¹Department of Nursing, Tan Tock Seng Hospital, Singapore, ²TB Control Unit, Tan Tock Seng Hospital, Singapore, ³STEP Registry, Tan Tock Seng Hospital, Singapore, ⁴Department of Respiratory Medicine, Tan Tock Seng Hospital, Singapore

Aim: 1) To identify patients' reasons for defaulting treatment; and 2) to study the demographic and social background of defaulters.

Methods: The survey was carried out by Tuberculosis Care Unit (TBCU) nurses who visited the homes of infectious PTB treatment defaulters, defined as patients who missed Directly Observed Therapy (DOT) at the polyclinic or their scheduled appointments at TBCU, to recall them for treatment. The reasons for defaulting were obtained from patients or relatives during the home visits. The social and demographic data of the defaulters were compared with that of PTB cases notified from 2001 to 2003 to the STEP registry.

Results: Ninety-nine defaulters' homes were visited between April 2002 to December 2003, of which contact was established with 59 defaulters or their family member(s). The main reasons for defaulting treatment were financial (39%) (of these, 70% were unemployed, and 70% were of non-Chinese ethnicity) and time constraints (28.8%) (of these, 88.2% were employed, half of whom held manual jobs). 63% of the defaulters lived in 3- to 4-room flats, and only 66% lived with their family. Non-Chinese were 3 times more likely to default, $P < 0.001$. Age and gender were not factors predictive for defaulting.

Conclusion: Financial difficulty was the commonest reason for defaulting DOT and clinic appointments. 69.6% who defaulted due to financial reasons were unemployed. Non-Chinese persons were 3 times more likely to default PTB treatment.

II106/NUR**Reducing Length of Stay of Patients Admitted with Pneumonia-Poster Presentation**P CHAN¹, FL SIN², FM KUTTY³, SF KOH¹, P VELUSAMY¹, L RAZAK⁴, S CHUA³¹Department of Nursing, Alexandra Hospital, Singapore, ²Department of Medicine, Alexandra Hospital, Singapore, ³Department of Pharmacy, Alexandra Hospital, Singapore, ⁴Medical Records Office, Alexandra Hospital, Singapore

Aim: Pneumonia pathway was implemented in November 2001. The team found that the average length of stay escalated from 7 to 10 days for the third quarter of 2003 and was higher compared to other institutions of 5-day length of stay. A multi-disciplinary clinical project team was formed with the target to reduce length of stay (LOS) to not more than 5 days in 50% of patients admitted with pneumonia without complications (DRG 171 & 172).

Methods: Data collected showed 5 key causes of long LOS. The following interventions were implemented: a) all new house officers were briefed on protocol and documentation; b) admitting doctors were allowed to order basic investigations only; c) pharmacists assisted in reminding doctors to oralise antibiotics as the patient's condition improved and fever subsided; d) ward nurses and case managers followed ward rounds, monitored and gently reinforced doctors to adhere to pathway.

Results: With the above interventions, 50% to 80% of patients achieved LOS of 5 days and below.

Conclusion: LOS was reduced from 10 days to 5 days and below. We have demonstrated that the involvement of a multi-disciplinary team approach using clinical pathway can reduce LOS without increasing patient morbidity.

II107/NUR**Better Late Than Never? A Report on Patients Attending a Multidisciplinary Osteoporosis Clinic**

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Aim: Osteoporosis is both a disease and a risk factor for fracture, with debilitating consequences. As our population ages, an estimated 17% of our elderly population is expected to have osteoporosis by 2030. Previous studies have demonstrated that osteoporosis is an under-recognised and under-treated problem. An osteoporosis clinic was started at Alexandra Hospital with the aim of using a multidisciplinary approach to effectively assess and treat osteoporosis.

Methods: We encouraged the referral of patients at risk of or suffering from osteoporosis to the clinic. We present the clinical profile of the patients ($n = 75$) attending the osteoporosis clinic from November 2002 to September 2003.

Results: Patients attending the clinic were predominantly women (92%) and consisted of 90% Chinese, 6% Malay and 4% Indian/other races. The median age of attendees was 68 years. The median T-score was -2.55 at the spine and -2.70 at the hip. Majority of the patients (69.2%) had at least 1 fracture. The most common fracture site was the spine (63%), followed by the hip (22%), other peripheral sites (11%) and both the hip and the spine (4%).

Conclusion: A large proportion of the patients attending the osteoporosis clinic had advanced osteoporosis with previous fractures. Greater awareness of the disease and earlier detection and treatment of osteoporosis should be advocated.

II108/NUR**Effectiveness of Nurse Clinician Case Manager and Telemedicine in the Management of Patients with Persistently Uncontrolled Diabetes Mellitus**M TA¹, BC LIM², HH TAN³, E CHAN⁴, K GU⁴, SY GOH², S LIANG⁴, CE TAN², SC LIM¹¹Diabetes Centre, Alexandra Hospital, Singapore, ²Diabetes Centre, Singapore General Hospital, Singapore, ³SingHealth Polyclinics, Singapore, ⁴Clinical Trial and Epidemiology Unit, Singapore

Aim: To determine the effectiveness of outpatient telemedicine based, diabetes nurse clinician supervised therapeutic education in subjects with persistently uncontrolled diabetes.

Methods: A total of 20 subjects with persistently uncontrolled diabetes (HbA1c $> 8.5\%$ for at least a year) were recruited from 2 tertiary diabetes centres. A diabetes nurse clinician was assigned as case manager to provide systematic and structured diabetes education via telemedicine (electronic mail) over a 6-month period. Interpretation and advice on self blood glucose

monitoring data was also communicated via email. Outcome measures include HbA1c and body weight at baseline, third month and sixth month. Repeat measurement technique is used to evaluate the time effect on the endpoints.

Results: There were 10 Chinese, 6 Indians and 4 Malays. The median age was 38 years (range, 19 to 52); median year of diagnosis was 8 years (range, 0.5 to 31). We found significant changes in weight and HbA1c by time ($P = 0.0105$ and $P < 0.0001$ respectively). Mean weight increased by time, while mean HbA1c decreases. Compared with baseline, the mean increase of weight at the first and the second follow-up visits were 0.84 kg (95% CI -0.16 to 1.83, $P = 0.096$) and 1.75 (95% CI 0.54 to 2.96, $P = 0.007$) respectively. Compared with baseline, the mean decrease of HbA1c at the first and the second follow-up visits were 1.26 (95% CI 0.81 to 1.71) and 1.19 (95% CI 0.69 to 1.70), respectively.

Conclusion: Telemedicine based therapeutic diabetes education may be effective in improving glycaemic control among subjects with persistently uncontrolled diabetes.

II109/NUR

A Simple Approach to Improve Indwelling Urinary Catheter Care

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Aim: There is a scientific basis that suggests a reduction in nosocomial infection rates could be achieved by optimising processes in the delivery of care to patients. The purpose of this project was to improve the insertion and day-to-day care of Indwelling Urinary Catheters (IDC).

Methods: The clinical quality improvement tool, Plan-Do-Study-Act Cycle was used. A pilot project was conducted in the Medical and Surgical wards. Tools were designed to measure the care associated with IDC insertion and maintenance. A denominator of device days and a numerator of catheter associated urinary tract infections (CAUTI), along with the CDC classification for symptomatic and asymptomatic CAUTI, were used.

Results: The results, obtained prospectively over 6 months, were analysed. After the introduction of the project, the CAUTI incidence rates for both wards decreased. Overall, the compliance rates to insertion and maintenance of IDCs improved. The majority of insertion non-compliance was associated with the aseptic hand-wash prior to IDC insertion and use of the sterile drape. During the maintenance phase, most non-compliance was associated with hand-hygiene before and after procedures and not wearing gloves when handling IDCs. Continuous feedback was given to staff and results were discussed with the Infection Control Committee. The measures implemented included reinforcement and on-site education. In addition, a training programme focusing on IDC insertion has commenced.

Conclusion: The project has improved the care associated with IDCs. The success of this project-based approach will be used to improve other processes involved in nosocomial infections.

II110/SC

Evaluation of Ease of Intubation with Glidescope or Macintosh Laryngoscope by Experienced Operators in Simulated Easy and Difficult Airways

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Aim: The new video-equipped laryngoscope, Glidescope® (GS), is developed for difficult airway management. We compared this device with the Macintosh laryngoscope (ML) in intubation of simulated grade 1 and 3 larynx by experienced anaesthetists.

Methods: In this double crossover study, 20 anaesthetists were randomised to use either the GS or ML first, and then randomised to intubate grade 1 or 3 larynx first. They were allowed 3 attempts to intubate in a maximum time of 360 sec in each of the 4 scenarios on the Human Patient Simulator.

Results: In the grade 1 scenario, success and ease of intubations were similar with both devices. Time taken was shorter with ML (mean 12.7 sec, SD 5.9 sec vs 19.0 sec, SD 9.7 sec, $P = 0.006$). Most of the anaesthetists chose ML (17/20) with 3 undecided. In the grade 3 scenario, there were more successful intubations with GS (20/20 vs 18/20, $P = 0.5$). Time taken was shorter for GS (mean 23.5 sec, SD 12.7 sec vs 70.5 sec, SD 101.2 sec, $P = 0.001$). The anaesthetists graded intubation easier with the GS (median easy vs intermediate, $P = 0.025$). More would choose the GS for the grade 3 scenario (12/20 vs 6/20).

Conclusion: In simulated grade 1 larynx, there was no advantage with GS, and intubating time was longer. In simulated grade 3 larynx, success rate and intubation time were better with the GS. We suggest further study of the GS in real patients.

II111/SC

Combined MIBI/US is the Modality of Choice: A Prospective Study Comparing MIBI, Ultrasound and Combined MIBI/US of the Neck in Hyperparathyroidism

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Aim: Ultrasound (US) and technetium-99m sestamibi scintigraphy (MIBI) are frequently used to detect abnormal parathyroid glands prior to surgical decision-making in hyperparathyroidism. This prospective study compares the efficacy of US, MIBI and combined MIBI/US in the detection of parathyroid adenoma or hyperplasia.

Methods: Twenty-nine patients with a mean age of 57 years were prospectively evaluated. Clinically, 15 patients were suspected of primary hyperparathyroidism, 7 of secondary hyperparathyroidism and 7 had recurrent disease. There were 113 parathyroid sites which were evaluated by MIBI, 94 sites by US and 94 sites by combined MIBI/US.

Results: The overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MIBI were 56.4%, 97.3%, 91.7% and 80.9%. For US, they were 50%, 96.9%, 88.2% and 80.5%. For combined MIBI/US, they were 63.3%, 96.9%, 90.5% and 84.9%. For primary hyperparathyroidism, the sensitivity rates of MIBI, US and combined MIBI/US were 78.6% and 63.6% and 90.9%. NPV was 93.6%, 90.7% and 97.5%, respectively. For secondary hyperparathyroidism, the sensitivity rates of MIBI, US and combined MIBI/US were 44.0%, 42.1% and 47.4%. NPV was 66.7%, 67.6% and 69.7%, respectively.

Conclusion: Combined MIBI/US imaging is better in detecting and in excluding parathyroid abnormality than either MIBI or US alone. In addition, although sensitivity, specificity, PPV and NPV are decreased in secondary hyperparathyroidism, combined MIBI/US continues to be better in detecting and in excluding disease than either MIBI or US alone.

II112/SC

MRI of Hip Pain: Prevalence and Mimickers of Radiographically Occult Hip Fractures

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Aim: Hip fracture, defined as fracture of the femoral neck region, is commonly diagnosed on radiographs. When it is minimally displaced or impacted, magnetic resonance imaging (MRI) is utilised to confirm the diagnosis. This study evaluates the prevalence of radiographically occult hip fractures and illustrates MRI appearance of some mimickers.

Methods: Fifty-five studies were performed in 54 patients (18 males, 36 females, mean age 55 years, range 13-94 years) with hip pain. MRIs were evaluated for the presence and location of bone or soft tissue injury.

Results: 25% (14/55) studies showed an acute fracture. Of these, 8 had a hip fracture and 6 had fractures in the ischium, pubis and/or sacrum. 15% (8/55) had other bone disease but not a fracture (5 avascular necrosis, 1 acute myeloid leukaemia, 1 lymphoma, 1 osteoarthritis). 16% (9/55) studies had muscle strain or grade 1 muscle tear without a fracture. 15% (8/55) had unrelated soft tissue disease (1 trochanteric bursitis, 1 pyomyositis, 3 paralabral cyst, 1 transient osteoporosis of the hip, 1 injection site oedema in the gluteal muscles, 1 non-specific hip effusion). 31% (17/55) studies had no

cause identified for hip pain.

Conclusion: Not everyone with hip pain has a hip fracture. Apart from diagnosing subtle fractures, MRI is also useful to demonstrate other bone or soft tissue conditions which cause hip pain. Because of the location of this wide range of mimickers, an imaging protocol which extends from pubic symphysis to sacrum is recommended.

II113/SC

Stereotactic Vacuum Breast Core Biopsy Using an Upright Mammographic Unit

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Aim: Vacuum-assisted core breast biopsy under stereotactic mammographic guidance is mostly performed in the prone position on a dedicated unit. However, the availability of add-on attachments allows the procedure to be done with lower cost and on pre-existing mammographic units. A review of our experience in TTSH with this method was performed.

Methods: From August 2001 to March 2004, there were 197 vacuum-assisted core biopsy procedures performed on 188 patients, using an 11G Mammotome biopsy device. The age of the study population ranged from 30 to 82 years. All technical details and complications were recorded. A follow-up phone interview was also conducted the next working day.

Results: The technical considerations with an upright mammographic unit for biopsy include a higher chance patient movement and limited infero-superior access. Patient movement can be minimised with adequate preparation and counselling, and by reducing the time taken for the procedure. The overall complication rate was 4% of all procedures. We had no major complications requiring hospital admission or surgical intervention. The common complication encountered was vasovagal reaction. All post-procedure bleeding was successfully controlled with direct pressure. None of the procedures needed to be aborted, giving a 100% technical success rate.

Conclusion: Stereotactic mammographic guidance for vacuum-assisted core biopsy is feasible with upright mammographic units. Awareness of technical differences compared to the prone unit along with good clinical practice will allow good technical success with a low complication rate.

II114/SC

Endovascular Repair of Aortic Aneurysm in Singapore General Hospital

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Aim: This study is a retrospective review of endovascular repair of aortic aneurysm in Singapore General Hospital.

Methods: From October 1998 to February 2002, 20 patients (17 males and 3 females with mean age of 72) with aortic aneurysm (17 infrarenal abdominal aortic aneurysms, 3 thoracic aneurysms) were treated with endovascular stent grafting. Aneurysm size ranges from 4.2 to 7.8 cm (mean 5.8 cm). Repairs were done with bifurcated stent graft in 13 patients, aorto-uni-iliac graft in 3 patients and tube stent graft in 3 patients. The patients were followed up clinically and with serial CT scans for mean of 11.7 months (range, 3 weeks to 35 months).

Results: Technical success was 95% (19 out of 20 patients). The single failure was due to inability to deliver the stent graft system secondary to tight iliac artery stenosis. Follow-up CT scans showed aneurysm sac shrinkage in 58.8% of the patients. Seven patients developed endoleak but 57% of them resolved spontaneously. One patient died within 30 days of the procedure. The death was not procedure-related.

Conclusion: Endovascular stent grafting is a safe and effective technique in the treatment of aortic aneurysm in our short-term review. However, long-term review is necessary to determine the durability of the technique.

II115/SC

Diagnostic Accuracy of Medical Officers in an Emergency Department

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Aim: To compare the effect of intradepartmental teaching and on-the-job supervision on diagnostic accuracy of medical officers at the beginning of a posting and towards the end of the posting.

Methods: A cohort of 13 medical officers posted to the Emergency Department of a tertiary teaching institution from November 2002 to May 2003 were selected. Ten consecutive admissions at the beginning and end of the posting by each medical officer were compared. Admission diagnostic accuracy was determined by classifying the admitting diagnoses as correct, non-specific, or outright wrong. Two independent assessors reviewed the case notes initially, and any differences in opinion were arbitrated by a third.

Results: Wilcoxon signed rank test was performed on the data. The mean number of correctly diagnosed cases was 6.08 at the beginning, and 6.85 at the end, with no significant difference detectable ($P = 0.197$). The mean number of non-specifically diagnosed cases was 1.69 at the beginning, and 2.31 at the end with no significant difference detected ($P = 0.241$). However, the number of wrongly diagnosed cases had decreased from a mean of 2.23 at the beginning to 0.85 at the end of the posting. This was statistically significant with $P = 0.036$.

Conclusion: There had been a significant reduction in the number of wrongly diagnosed cases. There was no statistically significant change in the number of correct or non-specific diagnoses.

II116/SC

The Presence of the Palmaris Longus: A Local Study

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Aim: The palmaris longus (PL), being functionally insignificant in flexion of the wrist, has been harvested for many reconstructive procedures. The prevalence of the palmaris longus in the local population has not been studied. Another study previously done has shown correlation between the absence of the PL and an incomplete superficial palmar arch (SPA). This is relevant in surgeries that involve a disruption of the vascular supply to the hand.

Methods: A total of 418 subjects was screened. The PL was visualised and palpated after the patient was asked to perform resisted flexion at the wrist with a clenched fist. The Standard Allen's test was used to determine if the palmar arch was complete or incomplete.

Results: Of the 418 subjects, 5.7% had an absent PL, of which 29.1% was bilateral. The SPA was complete in all bilaterally absent PL. The arch was incomplete in 17.6% of unilateral PL absence. Of the 24 hands with absent PL, 12.5% was associated with an incomplete SPA.

Conclusion: Our study of a sample of the local population places the presence of PL at 94.3%. Only 12.5% of our cases of absent PL had an incomplete palmar arch, which is lower than the 47% figure quoted in another such study done.

II117/SC

Dominant Versus Non-dominant Hand: A Difference in Maximum Grip Strength?

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Aim: A survey was conducted to establish the difference in maximum grip strength between the dominant and non-dominant hand in the local population.

Methods: The bilateral grip strengths of 402 normal subjects without any hand pathology were measured using a Jamar dynamometer. The population age ranged from 7 to 85 years and there were 255 female and 147 male subjects.

Results: The mean of the maximum grip strength of the dominant hand was 31.59 lb (± 10.57 lb) and that of the non-dominant hand was 29.34 lb (± 10.23 lb). The difference in the mean of the maximum grip strength between the dominant and non-dominant hand was 2.24 lb (95% CI: 1.90-2.58) which was statistically significant ($P < 0.05$). This was also the case when analysed within the female and male subgroups. Interestingly, the difference in the mean of the maximum grip strength between the dominant and non-dominant

hand was only statistically significant within the right hand dominant subjects ($P < 0.05$) and not within the left hand dominant subjects.

Conclusion: The maximum grip strength of the dominant hand was significantly higher when compared to the non-dominant hand. The difference was statistically significant ($P < 0.05$) within the male, female and right hand dominant subgroups, but not so in the left hand dominant subgroup. This data may be useful as a guide in the hand rehabilitation of patients.

II118/SC

Forearm-based Bier's Block is as Effective as a Conventional Bier's Block for Manipulation and Reduction of Distal Radius Fractures – A Prospective Randomised Controlled Trial

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Aim: The Bier's block is an effective regional anaesthesia technique. Modification of the block by placing a tourniquet on the forearm instead of the arm has been described. Advantages of the forearm tourniquet include preservation of the muscles of the long flexors and extensors of the hand, as well as longer tolerance of the tourniquet. This study compares the pain scores and complications of the forearm-based Bier's block versus the conventional Bier's block for the manipulation and reduction of distal radius fractures.

Methods: Patients being seen at the hospital's Emergency Department who were over the age of 16 and had a distal radius fracture that required manipulation and reduction were considered for inclusion. Patient biodata, mechanism of injury and radiological configuration of the fractures were collected. Pre-manipulation and manipulation pain scores were measured using a Visual Analogue Scale (VAS). Statistical analysis was performed using the Mann-Whitney U test.

Results: There were 17 patients in the conventional group and 14 patients in the forearm group. There was no difference in mean age, sex distribution, mechanism of injury or fracture configuration between the 2 groups. Statistical analysis showed no significant difference in the pre-manipulation pain scores, manipulation pain scores and difference between the 2 scores. There were no complications in either group.

Conclusion: The forearm-based Bier's block is as effective and safe as a conventional Bier's block.

II119/SC

Endoscopic Transnasal Approach for Clival Tumours

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Aim: Clival tumours are among the most challenging of skull base lesions to treat. The approaches proposed so far by other authors include anterior transfacial, frontal transnasal, or midface degloving approaches, lateral transtemporal or transpetrous approaches. These are invasive, traumatic and often disfiguring, and associated with morbidity, e.g. hearing loss. We decided to implement a new minimally invasive endoscopic transnasal approach for clival tumours.

Methods: Two patients, 1 with a clival chordoma, and another with a clival carcinoma, were treated with this novel method. Gross total resection was achieved.

Results: The patients recovered very quickly and well from the surgery, with no morbidity or complications. They were able to talk, eat, drink and walk on the same evening after the operation, and were discharged after only a few days.

Conclusion: The endoscopic transnasal approach for clival tumours holds great promise as a new minimally invasive, yet maximally effective surgical technique. However, familiarity and experience with endoscopic surgery is required for this method to be used safely.

II120/SC

Minimally Invasive Carpal Tunnel Decompression with Knifelight

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Aim: Carpal tunnel decompression is a common procedure in orthopaedic and neurosurgery. Endoscopic carpal tunnel decompression offers significant advantages over open surgical decompression. These advantages include a smaller incision, preservation of the overlying palmar fascia, palmaris longus, better post-surgical grip function, less pain and less scarring. However, compared to traditional surgical release, it requires more equipment and setup, such as endoscope, light source and monitor. We devised a new method using the Knifelight tool, which incorporates all the advantages of endoscopic release, but which does not require an endoscopic setup.

Methods: From April 2003 to June 2004, 20 consecutive patients with clinically symptomatic and electrophysiologically confirmed carpal tunnel syndrome were enrolled in our study. All underwent Knifelight release. A small 1-cm incision was made in the wrist crease. The transverse carpal ligament was identified, and a small opening made. The Knifelight was inserted, and the ligament release complete.

Results: All 20 patients experienced at least 90% improvement of their symptoms, and more than half had complete resolution. There were no complications related to the approach. The scar in the wrist crease was practically invisible after 2 months.

Conclusion: Our new Knifelight carpal tunnel release method offers a significant minimally invasive, simple and easy alternative to the current open surgical or endoscopic treatments.

II121/SC

Petrosal Keyhole Approach for Repair of Anterior Inferior Cerebellar Artery Aneurysm

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Aim: The prepontine space anterior to the brainstem is one of the most difficult areas to access in neurosurgery, due to the deep location, extremely narrow surgical corridors, and vital structures present (e.g. cranial nerves, brain stem vessels, brainstem). Surgical approaches to this region are usually complicated and invasive. Anterior inferior cerebellar artery (AICA) aneurysms are very rare, are located in this challenging region and are difficult to treat surgically. We report our experience with a minimally invasive petrosal keyhole approach for repair of AICA aneurysm.

Methods: A 63-year-old Chinese female presented in coma with an acute subarachnoid haemorrhage. Cerebral angiograms showed a large lower basilar trunk aneurysm and a left AICA aneurysm. The basilar trunk aneurysm was near totally coiled by neuroradiology, but the AICA aneurysm was uncoilable.

Results: A minimally invasive approach was used. A 6-cm retroauricular incision was made, and a targeted 3-cm diameter temporal bone drilling though the petrous was used to expose the AICA aneurysm at its junction with the basilar artery. The AICA aneurysm was successfully repaired. The patient recovered well, and eventually regained consciousness.

Conclusion: A rare AICA aneurysm has been successfully repaired with a minimally invasive petrosal keyhole approach.

II122/SC

Petrosal Keyhole Approach for Total Excision of a Giant Intracranial Epidermoid Involving the Posterior and Middle Fossa

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Aim: Intracranial epidermoid/dermoid lesions are rare, and difficult to remove totally. Giant epidermoids pose a formidable surgical challenge, and frequently involve multiple compartments. Their removal usually requires large or multiple craniotomies. We report our experience with a novel minimally invasive petrosal keyhole approach for total excision of a giant intracranial epidermoid.

Methods: A 59-year-old Chinese male presented with progressive right multiple cranial nerve palsies, right sided cerebellar signs, and ataxia. CT and MRI scans showed a giant epidermoid extending from the foramen magnum to the sphenoid ridge, compressing the brainstem anterior, laterally and posteriorly, elevating the floor of the third ventricle, and causing hydrocephalus.

A ventriculoperitoneal shunt was first inserted to relieve the hydrocephalus. A petrosal keyhole approach was used to remove the lesion.

Results: A minimally invasive 6-cm retroauricular incision was used. The petrous temporal bone was drilled to expose both the posterior and middle fossa. The superior petrosal sinus was ligated, and the tentorium was incised. The epidermoid was completely excised. The patient recovered rapidly and well from the procedure, with improvement in his neurological deficits.

Conclusion: The petrosal keyhole approach has been used to successfully excise a giant intracranial epidermoid involving multiple intracranial compartments.

II123/SC

Subtemporal Keyhole Approach for Lesions Involving the Cavernous Sinus

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Aim: Cavernous sinus lesions are extremely challenging and difficult to treat surgically. The neurosurgical approaches are complicated, invasive, and usually require large craniotomies and skull base approaches. We report our experience with a novel minimally invasive subtemporal keyhole approach for the treatment of cavernous sinus lesions.

Methods: From April 2003 to June 2004, 7 patients presented with multiple cranial nerve palsies due to cavernous sinus lesions. One patient had plasma cell granuloma, 1 had trigeminal schwannoma, 1 had haemangiopericytoma, 1 had skull base carcinoma, 1 had metastasis and 2 had meningioma. A subtemporal keyhole approach with a 5-cm pre-auricular incision was used. Limited anterior petrosectomy was performed. The cavernous sinus was opened, and the tumour dissected out.

Results: The patients recovered rapidly and well from this minimally invasive approach. Five patients even experienced improvement in their neurological deficits. None had any worsening of their neurological deficits.

Conclusion: The subtemporal keyhole approach is a novel, minimally invasive alternative to the more invasive established neurosurgical approaches for treatment of lesions in the cavernous sinus.

II124/SC

A New Transorbital Keyhole Approach for Intracranial Aneurysms

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Aim: The pterional craniotomy has been the workhorse for access to anterior circulation aneurysms for several decades. This requires head shaving, a large fronto-temporal incision, large fronto-temporal craniotomy and some brain retraction. We studied all the anterior circulation aneurysms admitted under the author's care from April 2003 to June 2004, to see if a new minimally invasive transorbital approach could be effectively used instead to treat these aneurysms.

Methods: Twenty-five patients who could benefit from this approach were identified. A 5-cm eyebrow incision was made, and a 3-cm orbitofrontal craniotomy was made. The orbital roof was drilled off to the superior orbital fissure. The dura was opened, and retraction was placed on the orbit rather than on the brain. The subarachnoid cisterns opened. The aneurysms were dissected out, and repaired with a neurosurgical clip.

Results: All the aneurysms were successfully repaired. These aneurysms were located at the anterior communicating artery, middle artery bifurcation, posterior communicating artery and internal carotid artery bifurcation. There were no complications or morbidity associated with the approach.

Conclusion: The transorbital eyebrow approach is a viable alternative to the traditional pterional approach for the treatment of aneurysms. Moreover, it offers the following advantages: 1) no hair shaving; 2) small incision, less pain and better cosmesis; 3) small craniotomy, less brain exposed; 4) no brain retraction; and 5) faster postoperative recovery.

II125/SC

Prevalence of Visual Impairment and Blindness Amongst Patients Attending a Geriatric Specialist Outpatient Clinic

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Aim: To assess the prevalence of visual impairment and blindness amongst elderly patients attending a geriatric specialist outpatient clinic and to ascertain the underlying causes of visual impairment and blindness.

Methods: We conducted opportunistic screening of visual acuity for 287 patients attending the Alexandra Hospital Geriatric Centre for non-eye related medical disorders. All subjects with visual acuity 6/18 or worse were referred for a complete ophthalmic evaluation to ascertain the underlying cause for visual impairment and blindness.

Results: The mean age of the 287 patients was 77 years (range, 53-102 years). Of these, 97 were males and 190 were females. Unilateral blindness (defined as visual acuity of worse than 6/60) was found in 16.4%, whereas bilateral blindness was seen in 5.2% of the patients. Unilateral and bilateral visual impairment (defined as vision of 6/18 to 6/60) was detected in 34.5% and 15.7% of the patients, respectively. Of the referred patients, 103 patients returned for a complete ophthalmic examination by an ophthalmologist. The underlying causes of visual impairment and blindness were cataract (62.1%), age-related macular degeneration (13.6%), glaucoma (9.7%) and diabetic retinopathy (7.8%).

Conclusion: The prevalence of visual impairment and blindness was high in elderly geriatric patients and the most common causes of these were cataract and age-related macular degeneration.

II126/SC

Quality of Life of Myopes Before and After Laser Assisted In situ Keratomileusis Lasik

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Aim: To determine the quality of life of myopes using utility value before and after laser assisted in situ keratomileusis (LASIK) and to identify factors that may influence any change in the quality of life after surgery.

Methods: 102 patients (202 eyes) with compound myopic astigmatism seen at The Eye Institute, National Healthcare Group, were interviewed in this prospective study. They were followed-up 3 months after their LASIK surgery. The time tradeoff (TTO) and standard gamble blindness (SGB) methods were applied for generating the pre- and post-LASIK utilities.

Results: Overall, there was an increase in patients' mean utilities after operation. The SGB utility showed 2.04% increase (0.98 to 1), while the TTO utility showed an improvement of 5.38% (0.92 to 0.98). While most patients' TTO utilities increased to 1 after LASIK (n = 44), 2 showed a decline in utilities from a pre-LASIK value of 1. A similar observation was made based on the standard gamble method. Based on the ANOVA model, lifestyle demand was the only significant variable associated with an improvement in post-LASIK TTO utilities (P = 0.047).

Conclusion: The practical difficulties associated with wearing spectacles and contact lens and the limitations they pose on sports and leisure activities have a significant effect on the quality of life of myopic patients. This study reports a better quality of life after LASIK as shown by higher mean post-LASIK utility values using both the time trade-off and standard gamble for blindness methods.

II127/SC

Concordance of Fellowship Vitreoretinal Specialists in Detecting Hard Exudates on Digital Ocular Fundus Images

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Aim: The use of digital fundus images from a non-mydiatic fundus camera to screen the ocular fundus for lesions of diabetic retinopathy is widely

practised. We aim to assess the agreement between 2 independent specialists in detecting hard exudates on digital fundus images of patients screened for diabetic retinopathy.

Methods: Two fellowship-trained retinal specialists independently read 1198 digital fundus images of 565 diabetic patients from a diabetic retinopathy-screening programme for the presence of hard exudates. Of these, 921 (77%) images were gradable. A third retinal specialist was an independent masked adjudicator.

Results: Overall agreement between the 2 specialists was 96% [$\kappa = 0.71$ (95% CI 0.59-0.84)] and 97% [$\kappa = 0.83$ (95% CI 0.74-0.91)] for the left and right eye, respectively. Concordance of the first specialist with the adjudicator was 97% [$\kappa = 0.81$ (95% CI 0.70-0.91)] for the left eye and 98% [$\kappa = 0.87$ (95% CI 0.79-0.95)] for the right eye. Concordance of the second specialist with the adjudicator was 96% [$\kappa = 0.71$ (95% CI 0.59-0.84)] for the left eye and 96% [$\kappa = 0.76$ (95% CI 0.66-0.86)] for the right eye.

Conclusion: A high degree of concordance between independent retinal specialists suggests uniformity in detecting hard exudates on digital fundus images obtained with a non-mydiatic fundus camera.

II128/SC

Giant Frontal Sinus Mucocele Presenting with a Subcutaneous Forehead Mass

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Aim: To report a patient with intracranial extension of a mucocele presenting primarily with a subcutaneous forehead mass and various ocular signs.

Methods: Case report.

Results: A 33-year-old Chinese female presented with blurring of the inferior visual field in the left eye, associated with periorbital swelling and a painless subcutaneous forehead mass above the affected eye. Clinical examination of the left eye revealed proptosis, periorbital swelling, partial ptosis and choroidal folds. An elongated, soft, subcutaneous mass was seen above the left eye. CT scan and MRI both showed a large, lobulated mass of mixed density within the left frontal sinus, measuring 2.6 x 4.5 cm. The mass had eroded the bone and extended intraorbitally and intracranially. A subcutaneous component was also seen but there were no other intracranial abnormalities. Exenteration of the frontal sinus, decompression of the mucocele and endoscopic removal of the mucosa and osteoma were performed. Intraoperatively, a left-sided frontal bone defect corresponded to the region of the soft tissue swelling. Histopathology showed a fibrous connective tissue cyst wall, partially lined by stratified squamous epithelium, with patchy chronic inflammation.

Conclusion: A subcutaneous soft-tissue mass may be the presenting complaint of a frontal mucocele. It is important to consider sinus disease as a differential diagnosis during the clinical workup of a patient with orbital disease presenting primarily with proptosis and ocular symptoms. A careful examination of the surrounding skin may suggest the diagnosis of sinus-related disease.

II129/SC

Non-surgical Management of Brow Ptosis Secondary to Facial Nerve Palsy

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Aim: To describe a non-surgical method of restoring eyebrow symmetry in facial nerve palsy.

Methods: Three patients with brow droop due to facial nerve palsy were chosen for the study. Three patients had recurrent brow ptosis despite direct brow lifts. A new brow was drawn superior to the existent brow to match the height of the opposite side. When the patient was satisfied with her appearance in the mirror and digital picture, the existing brow was shaved.

Results: All patients found the technique easy to perform. It also greatly enhanced their self-esteem.

Conclusion: In patients with brow ptosis, cosmetic makeup can be as effective as surgical techniques in restoring the brow symmetry after facial nerve palsy.

II130/SC

The Choice of Prophylactic Antibiotic Among Junior Ophthalmologists in the Management of Contact Lens Related Keratitis

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Aim: In developed countries, corneal ulcers occur mainly in contact lens wearers. The most common causative agent is *Pseudomonas aeruginosa* which is resistant to chloramphenicol eyedrop, but sensitive to gentamicin or quinolone such as ofloxacin. To prevent the occurrence of ulcers, prophylactic antibiotic for contact lens-related keratitis should target *P. aeruginosa*.

Methods: Telephone interviews of 50 on-call ophthalmologists in 50 eye casualty in the UK to find out which antibiotic(s) would be prescribed for a patient with contact lens-related keratitis (punctate corneal staining on fluorescence without ulcer).

Results: 80% of ophthalmologists recommended chloramphenicol eyedrops. 4% recommended gentamicin and 16% recommended ciprofloxacin or ofloxacin.

Conclusion: The prophylactic antibiotic prescribed by most on-call ophthalmologists was ineffective against *P. aeruginosa*, the most common cause of bacterial ulcer in contact lens wearers.

II131/SC

Peripheral Neurectomy of the Inferior Dental Nerve Involved in Trigeminal Neuralgia and its Ultra-Structural Features: A Case Report

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Aim: Trigeminal neuralgia is a painful neurological condition, treated essentially by medication (carbamazepine). Its aetiology is unclear, but central demyelination is a possible cause, leading to spontaneous impulses (Clin J Pain 2002;18:4-13). Interruption of this triggering mechanism is achievable with peripheral neurectomy. The aim of this paper is to present the ultra-structural features of a peripheral nerve involved in trigeminal neuralgia.

Methods: A 50-year-old female who suffered from trigeminal neuralgia affecting her right inferior dental nerve for the last 10 years was operated under general anaesthesia via an intra-oral approach. The inferior dental nerve was identified and avulsed, with release of its terminal branches at the mental foramen. A titanium screw was then inserted into the foramen.

Results: Under the electron microscope, there was evidence of marked proliferative and degenerative changes in the myelin sheath. Some axons with thickened myelin showed loss of periodicity and disruption of lamellae. There was an associated axonal degeneration in some areas. The G-ratio (axon diameter:axon diameter and myelin sheath) appeared to be smaller. There seemed to be an increase in collagen contents in the extra-cellular matrix.

Conclusion: These ultra-structural abnormalities showed comparable features mentioned in previous studies of trigeminal neuralgia involving the ganglion and nerve roots. However, many of these features could also be present in age changes. Therefore, immunohistochemical studies for sodium and potassium channels involved in axonal conduction need to be established to differentiate them.

II132/SC

Accuracy of Fine Needle Aspiration Cytology in the Evaluation of Thyroid Nodules

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Aim: The objective of this study is to evaluate the accuracy of fine needle aspiration cytology (FNAC) in the evaluation of thyroid nodules.

Methods: A retrospective review was conducted on all patients who underwent

preoperative FNAC and subsequent thyroidectomies for thyroid nodules between January 1997 and April 2004 by surgeons from the Department of Otolaryngology–Head and Neck Surgery at the National University Hospital. One hundred and ten patients fulfilled our criteria and their preoperative FNAC and final histology was collated and correlated. True-positive, true-negative, false-positive and false-negative values were derived and the corresponding sensitivity and specificity values calculated. Aspirates which were classified as suspicious on FNAC were considered to be malignant in the analysis.

Results: The final histological diagnosis confirmed 15 malignant neoplasms (13.6%) and 95 benign lesions (86.4%). Nine (8.2%) malignancies, 69 (62.7%) benign nodules and 13 (11.8%) suspicious nodules were diagnosed on FNAC. Nineteen (17.3%) smears were deemed to be non-diagnostic on FNAC. There were 13 true-positives (14.3%), 68 true-negatives (74.7%), 9 false-positives (9.9%), and 1 false-negative (1.1%). The sensitivity and specificity of FNAC in the detection of malignancy in thyroid nodules were hence, 92.9% and 88.3% respectively. The overall diagnostic accuracy of FNAC was 89.0%.

Conclusion: Fine needle aspiration cytology is an accurate tool in the assessment of thyroid nodules. Non-diagnostic and “suspicious for malignancy” lesions contribute significantly to its limitations. Hopefully, they can be minimised with good aspiration technique and ultrasound guided aspiration.

II133/SC

En Bloc Resection of the Temporal Bone for Cancer

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Aim: Malignancies involving the temporal bone are rare and are frequently advanced at the time of diagnosis. Treatment of these tumours usually requires a combination of modalities including surgery. While en bloc resection of the temporal bone during surgery is undoubtedly desirable, it is technically challenging. We describe our experience employing this approach.

Methods: Between February 1996 and May 2004, 9 patients underwent temporal bone resection for a variety of tumours. Six patients had primary malignancies involving the ear and temporal bone (5 squamous cell carcinomas and 1 malignant ceruminoma), while the remaining 3 patients had tumours which secondarily involved the temporal bone (1 osteosarcoma, 1 meningioma and 1 metastatic nasopharyngeal carcinoma). Five patients underwent lateral temporal bone resection preserving the facial nerve, while 4 patients underwent subtotal temporal bone resection. Surgery included neck dissection in 7, parotidectomy in 8, and reconstruction in all 9 patients. Five of the 6 patients who had never been irradiated previously received adjuvant post-operative radiotherapy.

Results: After a mean follow-up of 31 months, 7 patients are alive and disease free, 1 patient is alive with disease, and 1 patient has died of recurrent disease. Post-operative complications occurred in 4 patients (2 CSF leaks, 1 wound dehiscence and 1 major skin flap necrosis).

Conclusion: En bloc resection for malignancy involving the temporal bone is technically challenging. However, our experience is that this approach provides good local control and survival for a variety of tumours. The complications of surgery are acceptable.

II134/SC

Conundrum of the Cocoon Sclerosing Encapsulating Peritonitis Secondary to Peritoneal Dialysis

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Aim: Sclerosing encapsulating peritonitis (SEP), or “abdominal cocoon” as it is descriptively referred to, is an enigmatic condition of unknown aetiology. It is characterised by the diffuse appearance of marked sclerotic thickening of the peritoneal membrane resulting in intestinal obstruction. It is a rare but serious complication of continuous ambulatory peritoneal dialysis (CAPD).

Results: A 14-year-old male with a history of end stage renal failure on CAPD presented with symptoms of acute intestinal obstruction. A CT scan of the abdomen revealed distended small bowel loops clustered and displaced to the

right upper quadrant. The overlying peritoneum was markedly thickened and calcified. Laparotomy confirmed the diagnosis of SEP and he was treated with excision of the fibrocollagenous membrane. Postoperatively, he had prolonged ileus requiring parenteral nutritional support and peritoneal dialysis was restarted on postoperative day 10.

Conclusion: A high degree of cognizance is needed to facilitate diagnosis and treatment of this uncommon and potentially life-threatening condition.

II135/SC

Non-surgical Periodontal Treatment and High Sensitivity C-Reactive Protein Levels

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Aim: Diabetes has been found to be significantly associated with periodontal disease. Blood levels of high sensitivity C-reactive protein (HS CRP), which is an acute-phase protein, have been used as a systemic marker of different chronic inflammatory diseases, including periodontitis, and is suggested to be a means of predicting cardiac complications in diabetics. The aim of this study was to determine whether periodontal treatment causes improvement in systemic health, as measured by changes in serum HS CRP levels.

Methods: A single-blinded randomised clinical control trial was carried out on a multi-racial population of Singaporean diabetics with varied levels of metabolic control. The groups compared were oral hygiene instructions group, scaling plus oral hygiene instructions group and a non-treatment control group. Full mouth periodontal recordings made at 3 months were compared to baseline.

Results: A significant and sustained improvement in periodontal parameters was seen in both treatment and oral hygiene instruction groups, while the non-treatment group also showed minor improvement. No concurrent changes were noted in HS CRP levels, and no particular pattern was recognised.

Conclusion: Within the confines of this study, periodontal treatment does not seem to affect systemic health as measured by HS CRP levels. These findings also suggest that HS CRP may not be a sensitive systemic marker for overt infections like periodontitis, and/or the changes are not expressed over short periods of observation.

II136/SC

Influence of Anterior Occlusal Traits on Professional Assessment of Dental Aesthetics

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Aim: The perception of dental attractiveness has been showed to be influenced by the anterior dental features associated with malocclusions. The objective of this study was to determine the influence of anterior occlusal traits on professional assessment of dental aesthetics.

Methods: A sample of 21 orthodontists (mean age 34.5 ± 5.1 years) was asked to assess the dental aesthetics of 50 pairs of black and white photographs of teeth at maximum intercuspation and right buccal view. The 50 pairs of photographs represented a range of malocclusions with various degrees of dental crowding/spacing, overjet and overbite/openbite. The subjects were asked to mark the level of dental attractiveness on a 100 mm visual analogue scale with very unattractive (0 mm) and very attractive (100 mm) as the extremes. Each pair of photographs of the same dentition was scored by the principal investigators for overjet, overbite and crowding/spacing. Multiple linear regression was used for data analysis with the orthodontist's VAS scores as dependent variables, and principal investigators' scores for overjet, overbite and crowding/spacing as independent variables.

Results: The results showed that overjet and crowding/spacing was found to be a significant predictor ($P < 0.05$).

Conclusion: In conclusion, the assessment of dental aesthetics by orthodontists was significantly influenced by the degree of overjet and crowding/spacing while overbite did not play any significant roles.

II137/SC

Glycaemic Control and Responses to Periodontal Therapy in Diabetic Patients

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Aim: Glycaemic control of patients with diabetes has been shown to affect their periodontal disease severity. On the other hand, few intervention studies had examined the effect of periodontal therapy on glycaemic control. The aims of this study are to compare the effects of different treatment modalities on glycaemic control in patients with diabetes mellitus and to correlate the different periodontal parameters with glycaemic control.

Methods: Sixty-eight adult diabetic subjects were randomly assigned to 1 of the 3 groups: test (simple non-surgical periodontal therapy and oral hygiene), oral hygiene and control (no treatment) groups. For all subjects, full mouth periodontal charting and blood samples were collected at baseline, followed by 3 months.

Results: There was a significant improvement in plaque and bleeding scores of those in the oral hygiene and test groups; when compared with the control group (ANOVA P or <8%), no appreciable differences were found.

Conclusion: While simple periodontal therapy improves the periodontal status of subjects, it did not significantly improve glycaemic control in the short term.

II138/SC

Comparing Radiation Fractionation Schedules for Painful Bone Metastases: Are We Blinded to the Quality Of Randomised Evidence?

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Aim: The ideal fractionation schedule for palliation of painful bone metastases is controversial, with proponents for the use of both single and multiple fraction regimens. However, debate of the evidence has not adequately addressed the quality of randomised studies. Given the lack of quality assessment, our aim was to evaluate the quality of randomised studies using a validated checklist, and to discuss implications and future directions.

Methods: We performed a search for studies that could be reliably assessed using the validated quality assessment instrument. Independent assessors scored study quality using the instrument.

Results: The median quality score of the 17 identified randomised studies was 1 out of 5 (range, 0-3). The majority (71%) of points were awarded for the authors describing the study as "randomised". Method of randomisation and description of withdrawals and drop-outs scored poorly for most studies. None of the studies were awarded points for allocation concealment (blinding). The overall quality was deemed poor for 16 out of 17 (94%) studies.

Conclusion: The quality of published randomised evidence comparing efficacy of various fractionation regimens for palliation of bone metastases is uniformly poor, and the deficit should be acknowledged by those publishing meta-analyses. As poor quality studies bias results in uncertain ways, it is not possible to state with any confidence the superiority or equivalence of a particular fractionation schedule. Greater efforts are required by radiation oncology trial groups to improve quality, with a particular focus on developing methods of allocation concealment and comprehensively reporting results.

II139/SC

Combined Laparoendoscopic Intra-gastric Enucleation of a Stromal Tumour Near Cardia: Description of a New Technique RM SINGAPOREWALLA¹, HG BALADAS¹, DEL TAN²

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Aim: Our aim was to study the feasibility of a combined laparoendoscopic, minimally invasive technique for removal of a benign gastric stromal tumour located in the lesser curve close to the cardia.

Methods: A 52-year-old male presented with epigastric pain. Gastroscopy

showed a 3.8-cm smooth submucosal mass in the lesser curve close to the cardia. It was well circumscribed and submucosal on EUS. A diagnostic laparoscopy was performed by creating an open pneumoperitoneum through the umbilicus. A gastroscope was then inserted and three 5-mm ports were inserted transabdominally into the stomach after distending the lumen. The mucosa over the tumour was incised with diathermy and the tumour was enucleated by carefully peeling away the overlying mucosa and underlying muscle. An OGD snare was used to grasp the tumour and aid in retraction and final removal through the oral cavity. The gastric wall defect was closed intraluminally by laparoscopic suturing. Once haemostasis was secured, the 3 ports were removed and the stomach wall defects closed with continuous vicryl sutures.

Results: Gastrograffin study on the third postoperative day was normal. He made an uneventful recovery and remained asymptomatic at 6 months. The final histology was leiomyoma.

Conclusion: Enucleation of well-circumscribed GIST tumours near the cardia can be performed safely using this combined technique without the need for any gastric resection. This method is also more cost effective, as it does not require any staplers or intragastric balloon ports.

II140/SC

Non-occlusive Ischaemic Colitis Following Acute Ischaemic Limb Reperfusion

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Aim: Reperfusion syndrome is a well-recognised sequelae characterised by systemic complications and multi-organ dysfunction (MODS). We report the first case of a patient who developed non-occlusive ischaemic colitis following reperfusion of an acutely ischaemic lower limb, and review its possible pathogenetic mechanisms.

Methods: A 64-year-old male hypertensive chronic smoker presented with acute critical right lower limb ischaemia of 48 hours duration. Immediate exploration revealed thrombus in the right common femoral artery with poor backbleeding from profunda and superficial femoral vessels. A femoro-femoral crossover graft was performed with successful revascularisation. Postoperatively, the patient developed adult respiratory distress syndrome, hypotension, and acute renal failure with gross myoglobinuria. He complained of worsening lower abdominal pain on POD3 with distension and bleeding per rectum. Sigmoidoscopy revealed ischaemic colitis. Laparotomy confirmed nonviable colon from splenic flexure down to rectum, and resection with defunctioning colostomy was performed. No thrombus was found in the vascular pedicle, and subsequent histology confirmed acute coloproctitis. Postoperatively, the patient demonstrated resolution of MODS and return of normal bowel function with aggressive supportive therapy. A PubMed search using the keywords 'mesenteric ischaemia', 'reperfusion' and 'colitis' was made, and the literature reviewed.

Results: Rat models suggest that lower limb ischaemia-reperfusion causes significant decline in the splanchnic microcirculatory blood flow, disruption of gut mucosal tight junctions, elevation of systemic endotoxin concentrations, and cytokine activation initiating a systemic inflammatory response.

Conclusion: Non-occlusive ischaemic colitis is a rare but potentially fatal complication following attempted salvage in acute limb ischaemia reperfusion.

II141/SC

Fine Aspiration Cytology of Metastatic Carcinoid Tumour Report of a Case and Review of the Literature

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Aim: While carcinoid tumour is a relatively common neoplasm in surgical pathology, FNAC as a method of primary diagnosis has only been reported in the literature a few times. This study analyses FNAC as a useful and safe tool in the diagnosis of carcinoid tumours.

Methods: A 66-year-old Indian female presented with epigastric pain for investigation. Initial work-up included a FBC, LFT and OGD was normal. She subsequently underwent an ultrasound of her hepatobiliary system which showed 2 intra-abdominal masses in the epigastrium. CT and MRI were

performed to further evaluate the masses which found them to be within the omentum, not attached to surrounding viscera. FNA was performed, and was diagnosed as carcinoid tumour. Her urine 5HIAA and capsule endoscopy of her small bowel were normal. She underwent laparotomy and excision of the 2 carcinoid tumours.

Results: While FNA biopsy is a common procedure, the primary diagnosis of metastatic carcinoid tumour by FNA biopsy has only been reported in 7 case reports in the literature to our knowledge. The metastatic sites were widely varied. In our patient, the tumour did not cause her any significant symptoms after her initial admission. If tumours are not causing significant symptoms and a lesion is accessible to FNA biopsy, this may be the preferred method of diagnosis. Pretreatment of a patient with known carcinoid syndrome with antiserotonin and/or antihistamine medications may be advisable before performing FNA biopsy.

Conclusion: Fine-needle aspiration cytology can be a useful and safe tool in the diagnosis of carcinoid tumours, perhaps at times avoiding the need for surgery.

II142/SC

Pancreatic Anaplastic Cancer with Splenic Involvement Causing Splenic Infarct: A Case Report and Discussion

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Aim: Pancreatic cancer has the lowest 5-year survival rate of any cancer. Pancreatic body and tail cancer are notorious for delayed presentation as they are asymptomatic till the spread beyond the organ itself. We are reporting a case of pancreatic cancer with local invasion into spleen causing splenic infarct.

Methods: Our patient was admitted complaining of severe left hypochondrial pain of 1 day duration. History and clinical examination were suggestive of possible septic foci in the left upper quadrant. Inflammatory markers were elevated with normal tumour markers. CT abdomen and ultrasound revealed large heterogeneous mass in region of splenic hilum with invasion into spleen causing splenic infarct. OGD showed extrinsic compression. The patient was vaccinated against pneumococcus, meningococcus and *H. influenzae*. Distal pancreatectomy with splenectomy and partial adrenalectomy were performed on the patient on 19 May 2004. Intraoperative findings of the tumour involved distal pancreas, spleen and adrenal gland with infarction of spleen and metastasis in segments 2, 4 and 7 of liver. Postoperatively, the patient had an uneventful recovery and was discharged on the eighth day.

Results: Histology report showed poorly differentiated ductal adenocarcinoma of pancreas involving the splenic substance. Areas of necrosis were found within splenic parenchyma along with tumour cells. Omentum and adjacent lymphnodes were not involved.

Conclusion: Although pancreatic tail and body cancers are known and discussed in literature, it is very unusual for them to cause splenic infarcts and their association with adenocarcinomas of pancreas is virtually unheard of. Our literature search did not yield any results for associated splenic infarcts.

II143/SC

Pylephlebitis: A Case Report and Literature Review

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Aim: Pylephlebitis is defined as the infected thrombosis of the portal vein. It can complicate any intra-abdominal or pelvic infection that occurs in the region drained by the portal venous system, especially diverticulitis and appendicitis. We report a rare case of cholangitis complicated by pylephlebitis.

Methods: A 70-year-old Chinese woman presented with obstructive cholangitis. Intravenous meropenem was started. Blood culture grew on *Enterobacter cloacae*. On day 2, ultrasound of the hepatobiliary system showed shrunken gall bladder with multiple stones with a 2-cm stone in the distal CBD. PTC was performed as ERCP failed to cannulate the duct. Subsequent CT revealed thrombosis of the main portal vein and its branches. She was started on intravenous heparin. Even though the patient subsequently developed Ranson's 8 pancreatitis, secondary to venous congestion of the splenic veins, the patient responded well to maximal supportive therapy.

Results: The diagnosis of pylephlebitis was made from both the demonstration of portal vein thrombosis using CT and bacteraemia status of the patient. Cholangitis has been a rare cause of pylephlebitis. A review of the English literature only revealed 1 other case of cholangitis.

Conclusion: As a result of the low incidence of pylephlebitis, there has been a lack of controlled randomised trials. This limits most of the literature to case reports and case reviews.

II144/SC

Recurrent Small Bowel Obstruction Due to Phytobezoar CT Diagnosis

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Aim: Bowel obstruction by phytobezoars is uncommon and remains a diagnostic challenge. Their formation due to impaired gastric motility and/or poor mastication is well documented. We studied the usefulness of a preoperative CT scan in detecting intestinal bezoars. The role of blenderised diet in prevention was also studied.

Methods: A previously healthy 78-year-old edentulous Chinese male with a history of truncal vagotomy and pyloroplasty presented with acute small bowel obstruction. Conservative therapy failed requiring a laparotomy. A 5-cm ovoid phytobezoar was impacted in the jejunum. A week after discharge, he presented again with acute intestinal obstruction. A CT scan abdomen was done to try and identify the cause of the recurrent obstruction.

Results: CT showed an ovoid intraluminal mass in the distal small bowel with a mottled gas pattern. Proximal to this lesion bowel was dilated and distal to it was abruptly collapsed. These findings were pathognomic of an intestinal bezoar. A second laparotomy confirmed bowel occlusion by another phytobezoar which was surprisingly impacted 30-cm distal to the first enterotomy. A limited resection anastomosis was done. After recovery, the patient was started on blenderised diet regimen and has remained asymptomatic for the past 1 year.

Conclusion: Phytobezoar intestinal obstruction is an important differential in the elderly population. Preoperative CT scan findings are pathognomic and can clinch the diagnosis. It can avoid unnecessary delay in surgical treatment. Using a blenderised diet in high-risk patients may prevent recurrences.

II145/SC

Laparoscopic Adrenalectomy: An Overview of Our Experience at the National University Hospital in Singapore

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Aim: Laparoscopic adrenalectomy is the treatment of choice for adrenal tumours. We describe an overview of the development of laparoscopic adrenal surgery and our experience at the National University Hospital (NUH).

Methods: From 1995 to 2004, 56 adrenalectomies were performed at NUH. Of these, 29 were laparoscopic and the remaining open adrenalectomies (n = 27). About 80% of adrenalectomies had been accomplished laparoscopically in the last 5 years. The majority of laparoscopic adrenalectomies were performed for Conn's disease (n = 19; 66%). We further review our experience of laparoscopic adrenal surgery with pheochromocytoma (n = 4), Cushing's syndrome (n = 3) and compare the results with open adrenalectomy. One patient with bilateral Cushing's adenomas underwent successful total unilateral adrenalectomy and contralateral subtotal adrenalectomy.

Results: Comparing laparoscopic adrenalectomy for Conn's syndrome, Cushing's syndrome and pheochromocytomas, a significant reduction was observed in operative time and duration of hospital stay for the more straightforward Conn's patients. In Cushing's syndrome, patients undergoing adrenalectomy for adenomas fared better than those for bilateral hyperplastic disease. Analysis of pheochromocytomas showed that despite an increase in operative time with the laparoscopic technique compared to open surgery, the mean hospital stay dramatically reduced with the laparoscopic procedure (median 4 days) versus open adrenal surgery (median 10 days).

Conclusion: Laparoscopic adrenalectomy is now applied to a wide variety of adrenal tumours. The results achieved in our experience with laparoscopic

adrenal surgery are promising and in synchrony with published literature. The results affirm that laparoscopic adrenal surgery is the method of choice for organ confined adrenal disease.

II146/SC

Damage Control Surgery in Major Multiple Trauma Patients: Strategies to Improve Patient Outcome

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Aim: Damage control surgery involves resuscitative surgery and adjuvant interventions to control haemorrhage, prevent contamination and correct gross metabolic disturbances before definitive surgery. This study aims to assess outcome of the severely injured multitrauma patients undergoing damage control surgery, review their indications, the immediate post-operative strategies and outcome in these patients.

Methods: Data was obtained from TTSH's trauma registry between January 2002 to May 2004. Patients' age, sex, mechanism of injury, physiological data, injury types and grading, perioperative status, operative procedure(s), blood product requirements, ICU stay and outcome were recorded. Patients with an Injury Severity Score (ISS) of greater than 9 were included. Patients older than 75 years or who had significant intracranial injuries were excluded.

Results: This study included 83 patients. Sixty-three patients underwent definitive surgery (DS group). Eighteen patients underwent damage control surgery (DC group). Damage control included exploratory lapotomy, packing of the liver, retroperitoneum or pelvis, on-table hepatic or pelvic angiograms and embolisation, pelvic fixation, thoracotomy and packing of chest cavity, pericardial window, re-look laparotomy and thoracotomy. Mean ISS for the DS group was 23.7 and the DC group's was 39.4 ($P = 0.0001$). Mortality rates for the 2 groups were, however, not significantly different (DS group 30.15% and DC group 36.84%). Blood product requirements were significantly higher in the DC group ($>5L$) than the DS group ($<3L$). All DC group patients required ICU stay

Conclusion: Damage control surgery improves on the predicated outcome of major trauma patients undergoing surgery.

II147/SC

High Impact Falls: An Epidemiological Review and an Analysis of the Factors Influencing Outcome

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Aim: High impact falls cause significant injuries. It is the second commonest mechanism of injury in major trauma patients (ISS >9) seen at Tan Tock Seng Hospital. This study aims to define and analyse the epidemiological profiles of these patients. It also looks at whether factors like age, height of fall and intention of fall can predict the outcome.

Methods: Data of high impact fall patients seen at the ED from 2002 to 2003 was collected and analysed ($n = 159$).

Results: One hundred and eleven (70%) were below 62 years old. Fourteen died in the ED. Of the 145 admitted, 27 died subsequently. Thirty-one (74%) of those who died fell from a height ≥ 2 m. Of 118 survivors, only 57 (48%) fell from ≥ 2 m. The mean age of the survivors was 47.0 yrs whereas that of those who died was 50.6 years. Eighty-two (57%) of the 145 admitted underwent surgery and of these, 64 (78%) survived. Sixty-three (43%) of the 145 admitted were managed non-operatively and of these, 54 (86%) survived.

Conclusion: Falls from heights carry a significant morbidity and results in lengthy stays in the hospital. Moreover, the patients are often in their economically productive years. This study hopes to better define and understand the profiles of such patients. The authors hope that this study can help in the formulation of preventive and treatment strategies in managing high impact falls patients.

II148/SC

A Review of Breast Reconstruction Following Mastectomy in Tan Tock Seng Hospital

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Aim: To review the profile of patients who underwent breast reconstruction following mastectomy and to evaluate the outcome.

Methods: The medical records of 60 patients were reviewed retrospectively.

Results: Sixty patients underwent breast reconstruction following mastectomy from March 2000 to April 2004. The mean age of these women was 44.3 ± 7.1 years. Forty-nine were married, 9 were single and 2 were divorced. Fifty-three were Chinese, 4 Malay, 2 Indian and 1 was classified under "others". Eight were post-menopausal and 52 were pre-menopausal. The mean size of the tumour at the time of diagnosis was 25.2 ± 19.9 mm. Fifty (83.3%) had invasive carcinoma and 10 (16.7%) had ductal carcinoma in situ. Thirteen patients had multifocal disease. The patients who opted for breast reconstruction were significantly younger compared to those who underwent mastectomy alone. There was no difference in race, tumour size or histology between the 2 groups. One patient had a breast implant inserted while the rest had myocutaneous flap procedures, most commonly using a trans-rectus abdominis myocutaneous (TRAM) flap. There were no mortalities in this series. One patient developed a cerebrovascular accident, and another developed a flap haematoma which required surgical evacuation. Two patients developed local breast recurrences over a 4-year follow up period.

Conclusion: Younger patients are more likely to opt for breast reconstruction after mastectomy. The TRAM flap procedure is most commonly done in our centre and this is achieved with minimal morbidity.

II149/SC

Mycotic Abdominal Aneurysm Secondary to Melioidosis

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Aim: Melioidosis is a condition caused by *Burkholderia pseudomallei* and is endemic in Southeast Asia and Thailand. The incidence of melioidosis in Singapore is 7%. Melioidosis-causing mycotic aneurysm is rare and there are about 6 reported cases in literature. We present our experience with 2 cases of mycotic aneurysm secondary to melioidosis. The aim was to study and discuss in detail the aetiology, clinical presentation, investigations, and management of mycotic aneurysms secondary to melioidosis and its complications, and review its literature.

Methods: Both cases were Chinese males in their sixth decades with comorbidities of diabetes mellitus. They presented with fever and abdominal signs. CT scans showed leaking abdominal aneurysms and they underwent emergency laparotomy with ligation of aneurysms and extra-anatomic bypass and cholecystectomy.

Results: The first patient recovered well after rehabilitation. The second case was complicated by aorto-duodenal fistula. He underwent emergency re-exploration and repair of aorto-duodenal fistula but died postoperatively.

Conclusion: Melioidosis is a difficult condition to diagnose and treat. High degree of suspicion is required to diagnose this condition. CT scan is investigation of choice. Early intervention and aggressive surgical treatment is necessary and is the only chance for survival. Extra anatomic bypass is recommended as the procedure of choice. Long-term antibiotics are necessary. Early involvement of ID physicians would be helpful.

II150/SC

Laparoscopic Urology: The First 100 Cases in Tan Tock Seng Hospital

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Aim: We report our single institution experience in laparoscopic urology with regards to the type of surgery, length of operating time, stay in hospital and complications.

Methods: A total of 100 laparoscopic procedures were performed at our institution from August 2001 to May 2004 in 67 men and 33 women. These procedures included radical nephrectomy in 36, simple nephrectomy in 10, partial nephrectomy in 1, nephroureterectomy in 5, adrenalectomy in 6, laparoscopic radical prostatectomy in 3, robot-assisted (Da Vinci) radical prostatectomy in 5, pyeloplasty in 1, deroofting of renal cyst in 5, herniorrhaphy in 22 and miscellaneous procedures in 4. The length of operating time, complications and length of stay in hospital were evaluated.

Results: The median age of patients was 61. The median operating time for radical nephrectomy was 225 minutes, nephroureterectomy was 265 minutes, adrenalectomy was 160 minutes, bilateral herniorrhaphy was 112.5 minutes, robot-assisted radical prostatectomy was 270 minutes and laparoscopic radical prostatectomy was 560 minutes. Complications occurred in 12 (12%) of the patients. This included conversion to open surgery in 6 (6%) patients caused by intraoperative bleeding in 3 and adhesions in 3. There were no mortalities. The median postoperative stay in hospital for radical nephrectomy, nephroureterectomy and radical prostatectomy was 3 days, adrenalectomy was 2 days and herniorrhaphy was 1 day. There were 13 retroperitoneal and 62 transperitoneal approaches used.

Conclusion: Laparoscopic urology is fast replacing many open procedures, with all the advantages of minimally invasive surgery. Complication rates parallel those seen in other major laparoscopic centres with acceptable conversion rates.

II151/SC

Haemorrhagic Acalculous Cholecystitis Presenting as Overt Gastrointestinal Bleeding

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Aim: Haemorrhage is a known complication of cholecystitis, and has been reported in the presence and absence of gallstones. Clinical presentation is usually indistinguishable from that of acute cholecystitis, although there may be suggestive sonographic or CT findings.

Methods: We highlight a case of overt gastrointestinal bleeding secondary to haemorrhagic cholecystitis. The patient, an 82-year-old man, was admitted with symptoms of upper gastrointestinal bleeding and had endoscopic evaluation, which was normal.

Results: Due to persistent bleeding, a coeliac angiogram was performed which showed active contrast extravasation from the cystic artery. An embolisation was performed successfully and the patient had an uneventful subsequent cholecystectomy.

Conclusion: This case highlighted the advantages of angiography in the acute setting of overt gastrointestinal haemorrhage over exploratory laparotomy. It also illustrates that though haemorrhagic cholecystitis is a rare cause of upper gastrointestinal bleeding, it should be considered nevertheless when other common causes have been excluded.

II152/SC

Creating the Twin Peaks—Correction of Rare Synmastia

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Aim: We report a case of a 19-year-old Indian girl referred to us for reduction mammoplasty and correction of her synmastia.

Methods: There is a paucity of literature about this condition, with only 1 report of an attempt to correct this condition. Preoperative evaluation with ultrasonographic and mammographic imaging showed fatty tissues connecting the 2 breasts.

Results: We first performed a vertical scar reduction mammoplasty. Using the same access, we anchored the intermammary dermal/subdermal tissues to the sternal periosteum using interrupted PDS 1 sutures thus creating a sulcus. The postoperative recovery was uneventful with satisfactory cosmetic result.

Conclusion: A rare condition of synmastia in a 19-year-old girl is presented and our technique for correcting this is described.

II153/SC

Spontaneous Common Iliac Artery Rupture: A Fatal Presentation of Ehlers Danlos Syndrome Type IV

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Aim: Ehlers Danlos Syndrome Type IV is a rare autosomal dominant disorder of connective tissue predisposing to spontaneous rupture of large arteries. We describe our experience of a case of fatal spontaneous common iliac artery rupture.

Methods: A 31-year-old Chinese female presented with abdominal pain, hypotension and pallor, with tenderness in her right flank. Fluid resuscitation was followed by urgent abdominopelvic CT, which revealed a 10.8 x 10.7 cm right-sided retroperitoneal haematoma arising from dissection of the right common iliac artery. The patient developed refractory hypotension with progressive fullness in both flanks. Immediate transabdominal exploration of the retroperitoneum was met with uncontrolled bleeding from spontaneous transection of the right common iliac artery. The patient became asystolic and remained unresponsive despite prolonged cardiopulmonary resuscitation. Posthumously, the family revealed that the patient was diagnosed with Ehlers Danlos Syndrome Type IV in 1992 after genetic profiling. Two siblings had already encountered premature death of unknown causes. The literature was reviewed on this fatal complication.

Results: The incidence of Ehlers Danlos Syndrome Type IV approximates 1 in 625,000. Arising from mutation of COL3A1 gene, its vascular complications include spontaneous haemorrhage and dissection, aneurysm degeneration and fistula formation associated with mortality rates of 63%. Vascular tissue is often friable, making conventional repair near impossible.

Conclusion: Ehlers Danlos Syndrome Type IV is associated with significant mortality. Early suspicion of vascular complications of this rare disease and timely intervention can improve outcomes.

II154/SC

Inguinal Metastases from Mucinous Cystadenocarcinoma of the Pancreas: A Rare Presentation

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Aim: Cystic neoplasms of the pancreas are an uncommon entity. We report the first case of a patient with mucinous cystadenocarcinoma of the pancreas who presented with symptomatic inguinal metastases mistaken for an inguinal hernia, and discuss the diagnostic and therapeutic dilemmas of this condition.

Methods: A 72-year-old Chinese male presented with a 6-month history of a right inguinal swelling with pain and discharge. Surgical exploration revealed a 1.5-cm hard mass adherent to the posterior wall above the deep inguinal ring which was excised. Histology sections revealed metastatic mucin-secreting adenocarcinoma with central necrosis. Serum CA19-9 was normal. Abdominopelvic CT revealed a 6-cm cystic mass in the tail of pancreas with evidence of intraperitoneal and right inguinal metastases. The patient refused further oncological therapy, and passed away 4 months after diagnosis. A PubMed search using the key words 'mucin', 'cyst', and 'carcinoma pancreas' was performed and the literature reviewed.

Results: Frequently asymptomatic, mucinous cystic neoplasms of the pancreas affect predominantly males in the fifth to seventh decades. 65% occur in the pancreatic body and tail. Differentiation of these malignant lesions from inflammatory pseudocysts is mandatory in determining treatment, and MRI remains the diagnostic modality of choice. Extirpation of disease remains the only curative option, with 5-year survival rates of 50% to 75% after resection.

Conclusion: Mucinous cystadenocarcinoma of the pancreas is an uncommon entity which often remains asymptomatic. Inguinal metastases arising from this primary has not been previously reported, and is associated with a dismal outcome.

II155/SC

An Unusual Variant of Lemierre's Syndrome

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Aim: Lemierre's syndrome is characterised by an oropharyngeal infection leading to secondary septic thrombophlebitis of the internal jugular vein. It is classically anaerobic in origin and *Fusobacterium necrophorum* is the most common pathogen. We report an unusual variant of this syndrome caused by aerobic *Klebsiella pneumoniae* infection.

Methods: A 68-year-old male with uncontrolled diabetes presented with necrotising fascitis of left neck. No primary source of sepsis could be identified on examination. Neck x-rays revealed soft tissue gas and CT scan confirmed an extensive neck space infection with large pockets of gas and

thrombosis of the left internal jugular vein. A generous debridement with open drainage of the infected neck spaces was performed under antibiotic cover. Postoperatively, the wound was regularly assessed and debridement was repeated thrice. Prolonged course of antibiotics based on culture results was continued until healing was achieved.

Results: *Klebsiella* pneumonia was isolated and grown from the fluid, tissue and blood samples. No anaerobic organisms were isolated. Follow-up CT neck at 3 months showed resolution of the infection, although the vein remained obliterated. No primary focus of sepsis was detected elsewhere on investigations.

Conclusion: A review of literature shows that Lemierre's syndrome is essentially caused by an anaerobic oropharyngeal infection. Our case is unique in that it shows the classical radiological features of the syndrome in the absence of any identifiable oropharyngeal sepsis. The isolation of *Klebsiella* as a cause has not been previously reported.

II156/SC

Small Flat Rectal Adenoma Can Progress to Advanced Cancer in 3 Years

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Aim: Small flat colorectal adenomas with a high degree of dysplasia were hitherto thought to be exclusive to the Japanese population. Work in this department had previously described a prevalence of around 5% of flat colorectal adenomas in an unselected population presenting for elective colonoscopy. We now report a case of a man diagnosed with advanced rectal cancer 3 years after a small 5-mm flat rectal adenoma was picked up.

Methods: This is a retrospective case report reviewing all clinical, operative and pathology reports.

Results: This patient with a complex medical history of SLE, Klinefelter's and hypogonadism was referred for rectal bleeding in June 2000. Colonoscopy identified a small 5-mm flat rectal adenoma, which was confirmed on histology. The patient defaulted colonoscopic surveillance and presented in July 2003 with advanced rectal cancer. Abdominoperineal resection was performed. The pathology report was that of pT3N1. The patient underwent adjuvant chemoradiation.

Conclusion: This report highlights the importance of identifying flat colorectal lesions and destroying them either by hot biopsy or polypectomy. The rate of malignant transformation is worrying and supports previous reports of aggressiveness of flat colorectal cancers. Accumulation of such reports will add to the understanding of the morphogenesis of flat colorectal cancers.

II157/SC

Severe Acute Respiratory Syndrome (SARS) in Surgical Patients: A Diagnostic Dilemma

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Aim: The diagnosis of severe acute respiratory syndrome (SARS) in surgical patients can potentially be missed based on current World Health Organisation (WHO) case definitions.

Methods: We report a retrospective case series of 10 surgical inpatients diagnosed with SARS following an outbreak in the surgical wards. Patients were included if they fulfilled the WHO case definition of probable SARS, had an active surgical problem and were admitted to the surgical wards during the outbreak period. Clinical histories, laboratory investigations and radiological findings were reviewed and analysed.

Results: Mean age of the cohort was 57.6 years (range, 38-78 years). Nine patients had concomitant medical conditions. Three patients were in the post-operative period, the remaining had not undergone any surgical procedure. All patients presented with fever, 8 had accompanying respiratory symptoms. Lymphopenia and raised lactate dehydrogenase (LDH) was seen in 7 patients. Eight patients had positive bacterial cultures. Six patients tested positive for the SARS coronavirus using RT-PCR. Primary abnormality on chest radiograph was air-space opacification. Rapid progression of radiological changes was seen in 7 patients. Mortality rate for our cohort is 20%.

Conclusion: The diagnosis of SARS in surgical patients differs from that previously described in normal patients. An apparent cause of fever and

positive blood cultures cannot exclude a diagnosis of SARS. The current WHO case definition could result in delayed or missed diagnosis. Early isolation of febrile patients with positive contact history must be undertaken, even in the face of another identifiable cause.

II158/SC

Stricture at the Ileal Pouch-Anal Anastomosis after 2235 Cases of Restorative Proctocolectomy: Predictive Factors, Management, Functional Outcomes and Quality of Life

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Aim: Restorative proctocolectomy and ileal pouch-anal anastomosis (IPAA) has now become the preferred surgical procedure for mucosal ulcerative colitis and familial adenomatous polyposis. This study intends to determine the risk factors associated with stricture formation after IPAA performed at a tertiary institution and the functional outcomes.

Methods: Our Pelvic Pouch Database was queried for all patients who had undergone IPAA from 1983 to 2002. Information obtained included patient demographics and potential risk factors such as proximal diversion, anastomotic technique, pouch configuration and anastomotic complications. Functional outcomes of patients with strictures were compared with those without strictures.

Results: A total of 2235 patients underwent IPAA, of which 401 (17.9%) developed anastomotic strictures. Significant predisposing factors were use of proximal diversion, anastomotic complications such as dehiscence and enterocutaneous fistula and S-pouch creation ($P = 0.05$).

Conclusion: The incidence of anastomotic strictures after IPAA is 17.9%. Significant risk factors were proximal diversion, anastomotic complications and S-pouch creation. Most strictures are amenable to dilatation. Functional results for patients with strictures are similar to those without strictures.

II159/SC

Bilateral Breast Cancer

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Aim: To identify a group of patients who are at increased risk of developing bilateral breast cancer.

Methods: The medical records of 30 patients with bilateral breast cancer in the department from 2000 to 2003 were reviewed retrospectively.

Results: Thirty patients with bilateral breast cancer were seen in the department from 2000 to 2003. Sixteen had bilateral invasive carcinoma, 7 had ductal carcinoma in situ (DCIS) in the contralateral breast and 7 had bilateral DCIS. The mean age at diagnosis was 57.3 ± 12.0 years. Twenty-seven were Chinese and 3 Malays. Six had a positive family history of breast cancer. Twenty-two patients were found either on clinical examination or on imaging to have a tumour in the contralateral breast at first presentation. Eight patients were found to have a second tumour on surveillance, after a mean duration of 19 ± 14.9 months from the time of first diagnosis. The size and stage of the second tumour was not significantly different from that of the first. There was no significant difference in the age, race, parity, presenting symptoms, tumour size and stage at the time of diagnosis, or hormonal receptor status between those with bilateral cancer and those with unilateral cancer. On multivariate analysis, those with a positive family of breast cancer were found to be 4.9 times more likely to have bilateral cancers.

Conclusion: Patients with a positive family history therefore constitute a high-risk group and imaging of the contralateral breast is important even when no lumps are detected clinically.

II160/SC

Utility of Video Studies in Adults with Neurogenic Voiding Dysfunction

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Aim: Most adult voiding dysfunction can be evaluated with simple urological assessment. However, complex voiding dysfunction secondary to neurological

diseases requires more sophisticated studies such as video-urodynamic studies. We review our results of video-urodynamic studies for adults with neurogenic dysfunction. In particular, we ascertain whether it significantly assists in diagnosis and management of these patients.

Methods: A total of 106 video urodynamic studies were performed over a 24-month period, of which 79 were for adults with neurogenic voiding dysfunction. Sixty-six were males and 13 were females. Mean age was 50 (range, 17-80). Neurological deficits included spinal cord injuries (66), Transverse myelitis (4), syringomyelia (1), meningomyelocele (4) and cerebrovascular accidents (4). Pre-urodynamic management included indwelling catheter (11) and intermittent catheterisation (50). Twenty-seven had troublesome leakage.

Results: Abnormalities in cystometrogram included detrusor overactivity (26), poor compliance (28), decreased sensation (33) and leakage (47). Abnormalities in pressure-flow studies included detrusor acontractility/underactivity (55) and outlet obstruction (19). The anatomic sites of obstruction on fluoroscopy were external sphincter (8 detrusor sphincter dyssynergia) and prostate (11). Additionally, fluoroscopy revealed vesico-ureteric reflux (4), bladder diverticuli (6) and fir-tree appearance (12).

Conclusion: Video-urodynamics is able to provide urodynamic diagnoses of the neurogenic voiding dysfunction. It gives maximal as well as simultaneous data of the function and anatomy of the lower urinary tract, compared to standard urodynamic study. It is useful in evaluation of adults with neurogenic voiding dysfunction.

II161/SC

Abdominal Compartment Syndrome Complicating Emergent Repair of Ruptured Abdominal Aortic Aneurysm: Use of Silastic "Bogota Bag" Technique

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Aim: Abdominal compartment syndrome (ACS) occurs in 4% of patients with primary closure following ruptured abdominal aortic aneurysm (AAA) repair, and significantly contributes to rapid multi-organ failure. We report a case managed successfully with abdominal decompression and temporary coverage with a silastic "Bogota bag", and discuss management options of this unusual complication.

Methods: A 69-year-old Chinese male hypertensive presented to the emergency department with a ruptured 9.5-cm infrarenal AAA. Immediate exploration and repair was performed using a Dacron graft via a midline laparotomy incision. Primary abdominal closure was unsuccessful due to intestinal edema following aggressive resuscitation, and the wound was covered with a silastic "Bogota bag". Relook laparotomy undertaken 12 hours later arrested bleeding from lumbar vessels with successful abdominal closure en masse after documenting intrabdominal pressure (IAP) of 14 mmHg. The patient subsequently developed ACS with refractory hypotension, oliguria and renal failure despite inotropic support and IAP of 20 mmHg.

Results: Urgent abdominal decompression and Bogota bag coverage was followed by swift restoration of multi-organ function. Delayed primary closure was performed 48 hours later without adverse sequelae and the patient survived. PubMed search using keywords "aortic aneurysm" and "abdominal compartment syndrome" was performed.

Conclusion: Prompt recognition of raised IAP and early decompression improves survival and reverses organ dysfunction caused by ACS. Delayed primary closure following emergent AAA repair does not significantly increase risk of graft infection. Plastic sheets and mesh materials also permit tension-free peritoneal coverage.

II162/SC

Cost Effectiveness of Early Versus Interval Cholecystectomy for Acute Cholecystitis in a Tertiary Institution in Singapore

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Aim: Controversy exists as to whether acute cholecystitis should be surgically treated with early or interval cholecystectomy. The clinical results and cost effectiveness of early versus interval cholecystectomy was compared.

Methods: A retrospective review of the clinical records of all subsidised

patients treated for acute cholecystitis in our department between January 2002 and May 2003 was conducted. These patients had their diagnoses confirmed radiologically and were initially started on wide-spectrum antibiotics. Those who did not respond clinically were treated with early cholecystectomy within the same hospital admission. The patients with clinical improvement were treated with interval cholecystectomy after 6 weeks. Parameters reviewed included total duration of hospital stay, complications and total hospital bills incurred.

Results: Forty-six patients were recruited in this study, of which 36 had interval cholecystectomy (Group A) and 10 had early cholecystectomy (Group B). The total bill incurred by the Group A was S\$7487 versus S\$6170 for Group B. The total length of stay in hospital for Group A was 11.2 days versus 6.5 days in Group B. There was no significant difference in morbidity rates between the 2 groups.

Conclusion: Total costs of treatment and length of stay were on average higher in patients who underwent an interval cholecystectomy versus an early cholecystectomy. The outcomes between the 2 groups were similar. Therefore, early cholecystectomy is a more cost-effective method of treatment for acute cholecystitis.

II163/SC

Acute Renal Failure Complicating Bilateral Retrograde Pyelography and Ureteropyeloscopy: A Case for Staged Endoscopic Evaluation for Upper Tract Haematuria

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Aim: Acute renal failure complicating retrograde pyelography (RPG) is an extremely rare phenomenon. We report the first case of ARF complicating simultaneous bilateral RPG and ureteropyeloscopy (UPS).

Methods: A 42-year-old Caucasian man presented with a 4-month history of right loin colic and gross haematuria. Renal function was normal and CT evaluation of the urinary tract was unremarkable. In view of persistent haematuria, the upper tracts were evaluated under general anaesthesia with simultaneous RPG and UPS. Biopsy of a polypoidal mass in the right lower renal calyx confirmed nephrogenic adenoma. Postoperatively, the patient developed severe bilateral flank pain and complete anuria. Serum creatinine rose to 668 µmol/ml over 2 days with minimal urine output despite intravenous diuretics. No obstruction was identified on serial ultrasonography and CT imaging. Massive diuresis in excess of 4 L/day followed with return of normal renal function by the seventh postoperative day. The patient remains symptom-free with normal renal function 4 months after endoscopic evaluation.

Results: Eight cases of ARF following RPG have been reported to date, but none involving simultaneous endoscopic instrumentation. Postulated mechanisms include bilateral ureteric obstruction and pyelotubular or pyelolymphatic reabsorption of contrast media. Mean duration of anuria was 4.2 days, with full recovery of renal function after 5 to 30 days.

Conclusion: ARF following RPG and UPS is a rare but potentially devastating complication. We advocate a staged approach to endoscopic and radiologic evaluation of upper tract haematuria.

II164/SC

Nephrogenic Adenoma Arising De Novo from the Renal Pelvis

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Aim: Nephrogenic adenomas are uncommon benign metaplastic lesions of the urinary tract. Up to 75% of these occur in the bladder, and the overwhelming majority are associated with urothelial irritation. We report a rare case of nephrogenic adenoma that arose de novo from the renal pelvis, and review the literature on the clinical profile of this rare entity.

Methods: A 42-year-old Caucasian male with no previous history presented with intermittent right loin to groin pain associated with gross haematuria and passage of clots. His symptoms persisted despite normal CT urography. A 1-cm exophytic polyp in the lower calyx of the right kidney was identified on bilateral retrograde pyelography and ureteropyeloscopy, with normal anatomy of both upper tracts. Histology demonstrated papillary renal tissue lined with cuboidal epithelium displaying tubule formation. Immunostaining was positive for CK7 and negative for WT-1.

Results: The patient developed transient acute renal failure following endourological upper tract evaluation, but recovered full renal function and has remained symptom-free for over 6 months. To date, only 24 cases (age 9-67 years) have been described in 3 reports. There is a male predominance (2:1). Identified aetiologies include structural abnormalities (2), previous surgery (1), chronic irritation (2) and urolithiasis (19).

Conclusion: Nephrogenic adenomas arising from the renal pelvis are extremely rare. Surgical options include endoscopic laser ablation or nephrectomy should the patient develop refractory haematuria or pain.

II165/SC

Dermal Vasculitis from Synchronous Renal and Bladder Transitional Cell Carcinoma Metastases to Skin Mimicking Carcinoma Erysipelatoides

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Aim: Cutaneous metastases from bladder account for 2% of extravesical disease. We report the first case of a patient with dermal vasculitis secondary to inflammatory cutaneous metastases from synchronous renal and bladder transitional cell carcinoma, mimicking carcinoma erysipelatoides.

Methods: A 62-year-old Chinese non-smoker male was diagnosed with Grade 2 transitional cell carcinoma in 1996 after presenting with gross haematuria. He developed multiple recurrences of superficial bladder TCC from 1997 to 2003, which were treated with repeated transurethral resection and postoperative intravesical BCG (Bacillus Calmette-Guerin). Radical cystectomy and right nephroureterectomy with urinary diversion via a Wallace Type 1 ileal conduit was performed in July 2003. Histology revealed synchronous Grade 3 transitional cell carcinoma in the bladder and right ureter, with transmural and vascular extension of disease. A month later, the patient returned with fever and a 2-cm inflammatory mass in the lower abdominal wound.

Results: Abdominopelvic CT showed a 3.5-cm mass in the anterior abdominal wall. He developed a painless warm erysipeloid rash over the suprapubic region. Incisional biopsy confirmed poorly differentiated carcinoma. Histology of the dermal rash revealed vasculitis with absence of tumour cells in dermal lymphatics. The patient declined further treatment with chemotherapy and topical steroids, and passed away 9 months from the onset of cutaneous metastases.

Conclusion: Dermal vasculitis secondary to cutaneous metastases from transitional cell carcinoma of the urinary tract is extremely rare and associated with a dismal prognosis. Palliative chemotherapy remains the mainstay of treatment.

II166/SC

Surgical Complications of Tenckhoff Catheters Used in Continuous Ambulatory Peritoneal Dialysis

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Aim: To determine the surgical complications associated with Tenckhoff catheters

Methods: A retrospective review of consecutively implanted catheters between 2000 and 2003 was performed. All catheters used were double-cuffed, pig-tail Tenckhoff catheters. They were inserted by one of the urological access team surgeons under local anaesthesia, using an open lateral transrectus muscle technique via a paramedian transverse incision. All patients were reviewed until time of transfer to haemodialysis, death, or if alive and receiving CAPD, December 2003.

Results: Of the patients for CAPD, 123 (89%) had no previous renal replacement therapy and 16 (11%) were transferred from haemodialysis. 82% of the patients had multiple co-morbidities, but day surgery for utilised for 41% of the cases. There were no significant intraoperative complications and no deaths were directly attributable to catheter placement. Early

complications included 30 cases of wound or exit site infection, 11 cases of wound haematoma (4%), and 12 cases of malposition or poor flow (7%). There was a significant association of early complications with reinsertions, previous abdominal surgery, and long operative duration (>60 minutes). CAPD peritonitis was the most common late complication, accounting for the removal of 16% of the catheters inserted. Mean catheter survival time was 43 months with no significant difference between diabetic and non-diabetic patients.

Conclusion: Tenckhoff catheter insertion under local anaesthesia is safe and for selected patients, a suitable day case procedure. However, early and late complications still result in significant morbidities. Newer techniques may facilitate the decrease of these complications.

II167/SC

Initial Experience of Laparoscopy on Complicated Tenckhoff Catheter Insertion and Revision

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Aim: Tenckhoff catheters are an important access for continuous ambulatory peritoneal dialysis. Though Tenckhoff catheter insertion is a simple procedure, it is not without complications. Poor flow/blockage may warrant "blind" surgical revision/repositioning. With the advent of laparoscopy, surgeons have a window of "vision" for diagnostic and therapeutic revision of complicated Tenckhoff catheters.

Methods: Malfunctioning and potentially complicated cases of Tenckhoff catheter insertion due to previous abdominal surgeries were listed for laparoscopy. The initial experiences of laparoscopy for Tenckhoff catheters are described.

Results: First was a malfunctioning Tenckhoff with poor drainage. Laparoscopy confirmed radiographic findings of migrated tip of catheter to the upper abdomen because of pelvic adhesions. Problem corrected by relocating the Tenckhoff catheter tip into an identifiable space in the pelvis. The second case of laparoscopy showed Tenckhoff catheter wrapped by omentum and adherent to the anterior abdominal wall. Omentum was dissected off the Tenckhoff catheter and adhesiolysis done. The third laparoscopy case was to assist insertion of a Tenckhoff catheter. A high risk for multiple post-operative adhesions was entertained because of numerous previous abdominal surgeries. Laparoscopic evaluation showed minimal adhesions and a Tenckhoff catheter was inserted. All cases exhibited good postoperative functional use of Tenckhoff catheters.

Conclusion: Laparoscopy is a good adjunctive tool for revision of malfunctioning Tenckhoff catheters. It helps identify the causes for Tenckhoff catheter malfunction and its subsequent correction. Laparoscopy is also an option for potentially complicated cases of Tenckhoff catheter insertion. It aids in proper positioning of the Tenckhoff catheter tip.

II169/SC

Initial Experience on Laparoscopic Urology at the National University Hospital, Singapore

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Aim: Minimally invasive surgery is a noble evolving practice from conventional open surgery. Through various "keyhole" incisions, surgeons operate on patients without having to make unavoidable lengthy incisions which may compromise overall patient recovery and satisfaction. We report our first year experience on laparoscopic surgery in urology.

Methods: Between June 2003 to June 2004, 12 cases of various laparoscopic cases done by the Department of Urology of the National University Hospital. A retrospective review of all the cases to obtain the various intra and post-operative data.

Results: To date we have performed 2 cases of laparoscopic nephrectomy, 1 case of laparoscopic nephroureterectomy, 2 cases of laparoscopic excision of renal cysts, 2 cases of laparoscopic orchidectomy, 1 laparoscopic ureterolithotomy, 1 laparoscopic kidney biopsy, 2 laparoscopic revision of malfunctioning Tenckhoff catheters and 1 laparoscopic insertion of Tenckhoff

catheter. No cases warranted conversion to open urological surgery. All cases were performed via transperitoneal route except for 1 retroperitoneal laparoscopic ureterolithotomy. All cases showed good patient tolerability and satisfaction with no peri-operative untoward complications. Hospital stay varied on a case to case basis but generally showed a shorter length of stay compared to its counterpart conventional open urological surgery. Average hospital stay is 3.75 ± 2.56 days. Average operating time is 153.75 ± 102.74 minutes.

Conclusion: Laparoscopic urological surgery has shown good patient tolerability. Our initial experience with laparoscopic surgery in urology appears promising. With the increasing experience, we would like to further extend the scope of surgery to laparoscopic pelvic surgery.

II170/SC

Application of Object Oriented Methodology for Case Reasoning Data Capture

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Aim: A computerised on-line diagnostic system (NUS TMD v1.1) was developed to address the time lag between RDC/TMD (Research Diagnostic Criteria for Temporomandibular Disorders) administration and generation of clinical diagnosis/psychosocial profiles. The RDC/TMD is not comprehensive due to the lack of data for rarer disorders. The current project aims to expand the clinical utility of NUS TMD v1.1 via incorporation of case-based diagnosis using object oriented methodology.

Methods: Object Oriented Programming (OOP) entails building of independent pieces of code which interact with one another. Microsoft Visual C++ 6.0 was used to develop extension modules incorporating the diagnostic classification of the American Academy of Orofacial Pain. Items included diagnosis for cranial bones, temporomandibular joints and muscle disorders. The program was tailored for deployment on Windows XP and tablet PCs which facilitated data entry by patients and clinicians. Data acquired was automatically archived for statistical analysis and use for case-based reasoning diagnostic criteria development. User acceptance testing (UAT) was subsequently performed.

Results: UAT on random data set confirmed that the new facilities developed satisfied the defined user requirement specifications. Data exported from the extension modules was automatically archived upon program closure. Data imported from TMD v1.1 were also verified.

Conclusion: The enhanced software (NUS TMDv2.0) was backward compatible. Ease of data collection, export and import facilitates collaborative multi-centre clinical trials. In addition to diagnosis, OOP could also be used to facilitate the gathering of treatment and other variables for use with case-based reasoning.

II171/SL

The Influence of Bone Marrow Derived Mesenchymal Stromal Cells on Rate of Tendon Healing in Rabbits

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Aim: Tendon injuries are common and have significant morbidity and socioeconomic impact. Previous studies have shown that the use of bone marrow derived mesenchymal stem cells (bMSC) in tendon defect repair improved biomechanical properties of the regenerated tendon. This study investigates the influence of bMSC on the healing of the primarily repaired tendon.

Methods: The gastrocnemius tendon of adult NZW rabbits was completely lacerated and repaired. For the treated group, bMSCs were introduced into the proximal and distal ends of the repaired tendon as well as around the repair site. The contralateral tendon served as the control and underwent the same procedure except without bMSCs. At 1 and 3 weeks, tendons were harvested for histological analysis. Standard H&E staining and immunohistochemical analysis of collagen I and III were used to observe healing.

Results: H&E stains revealed thicker paratenon in some of the treated tendons as compared to the control in the first week. Collagen I staining showed that at 3 weeks, collagen fibres in the treated tendons ($n = 5$) appeared to be better organised around the repair site as compared to the controls ($n = 5$). The fibres were more uniformly arranged and oriented according to the line of force.

Conclusion: bMSCs have some influence on the healing of primary repaired tendons. Histological findings show increased cellular events in the paratenon region and better organisation of collagen I fibres around the repair site. Whether these changes result in improved tendon strength during the healing phase is being addressed in current biomechanical studies.

II172/SL

In Silico Approach of Screening for Candidate Nucleic Acid and Protein Markers for Epithelial Ovarian Cancer Using Published Microarray Expression Data

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Aim: Microarray-based in-silico studies are ideal to study genes involved in oncogenesis pathways. We used an in-silico approach to identify candidate biomarkers for early detection of epithelial ovarian cancer (EOC) based on microarray array expression data with emphasis on regulatory elements.

Methods: Using in-silico approach we tried to identify genes involved in (1) membrane damage, (2) apoptosis and (3) disruption of cytoskeleton plasticity which is key to oncogenesis. Promoter content of target genes were analysed using Match program (Biobase and TRANSFAC Professional database ver7.4) from 14,333 human promoters and 12,000 human non-promoters (our internal human promoter database).

Results: Distinct subset of genes belonging to matrix metalloproteinases (MMP7,9,10,12) involved in catabolism, degradation of the extracellular matrix and lysis of membrane, and keratin subgroups (KRT8,13,18) involved in structural integrity of epithelial cells, cytoskeleton (membrane) maintenance and epidermis development were identified. Promoter analysis of 19 highly over-expressed genes of EOC contrasted to non-promoter sequences showed that several transcription factors (TF) (individually as well as in combinations) play a major role in controlling expression of these genes. We found strikingly different sets of TF binding sites that are present in 1 subgroup but not in the other and vice-versa. Genes and TFs identified during this study have the potential to be applied as clinical biomarkers for EOC.

Conclusion: In-silico approach is suitable for identification of potential target biomarkers since the data output is based gene regulatory elements which control the expression of such genes during the specific diseased state.

II173/SL

The Aneuploidy Screen Using Fluorescence In situ Hybridisation (FISH) for Prenatal Detection of Chromosomal Anomalies – A One Day Analysis on Chorionic Villus Biopsies, Amniotic Fluids and Fetal Bloods

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Aim: Rapidly advancing technology has made prenatal screening and diagnostic tests for expectant women more efficient and precise. With higher resolution ultrasound machines, abnormal fetal markers are noticed at earlier gestational ages. In such situations, parental anxiety runs very high and the demand for immediate resolution arises. With the knowledge that the risk for certain chromosome anomalies increases with abnormal ultrasound and triple test results, karyotyping is advised. The procedures available for prenatal diagnosis are chorionic villus sampling, amniocentesis and fetal blood sampling. A chromosomal karyotype takes between 4 and 10 days. However, FISH takes only 24 hours irrespective of sample type.

Methods: This study presents the results of 100 samples with abnormal ultrasound markers and FISH testing confirmed by traditional cytogenetic karyotyping. Vysis (USA) probes were used for FISH for chromosomes 13, 18, 21, X and Y on uncultured cells of all the above samples. At least 100 nuclei were counted for each DNA probe.

Results: Of the total samples referred for FISH, 31.5% had an abnormal karyotype. All were successfully detected by FISH.

Conclusion: Fluorescence in situ hybridisation (FISH) has proven itself to be a sensitive and reliable test in the identification of aneuploidies of chromosomes 13, 18, 21, X and Y. This test is very helpful to parents and gives them more time to make informed decisions in planning the future course of the pregnancy.

II174/SL

Haemostatic Status and Fibrinolytic Response Potential at Different Phases of the Menstrual Cycle

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Aim: To study the natural hormonal influence on haemostasis and fibrinolytic response at different phases of the menstrual cycle. The influence of natural hormones during the menstrual cycle on haemostasis has been varied.

Methods: Coagulation activation, factors and inhibitors including platelet function and fibrinolytic variables were determined in 30 normal healthy women volunteers aged 18 to 38 years. Coagulation status was determined using the computerised Thrombelastograph. Blood samplings were performed at different phases of the menstrual cycle: menstruation (day 1-3), follicular (day 5-9), mid-cycle (day 10-14), and luteal (day 21-26). The women were later divided into those with normal and overweight according to the WHO classification and the data re-analysed.

Results: No statistical significant differences in the haemostatic parameters studied between the phases of the menstrual cycle except for a reduced D-dimer level at mid-cycle was seen. Significant fibrinolytic response seen was not significantly different between the menstrual phases. In overweight women (n = 8), elevated t-PA antigen, PAI-1 levels except at menstruation, and total protein S except at follicular phase were observed together, with increased plasminogen level only at luteal phase. Similar significant fibrinolytic responses were seen in both normal and overweight women.

Conclusion: The study demonstrated that systemic haemostasis was not influenced by normal hormonal changes occurring during the menstrual cycle except for an associated reduced fibrinolytic state at mid-cycle. The haemostatic system in this small group of healthy overweight women appeared to be physiologically compromised.

II175/SL

Evaluation of Biofilm Formation on Soft Monthly Disposable Contact Lenses Among Healthy Individuals

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Aim: Biofilm, a functional consortium of micro-organisms, can be found on surfaces of many medical devices and cause severe infection. This study aims to study the presence of biofilm on soft, monthly disposable contact lenses among healthy individuals.

Methods: This is a prospective study. Using block randomisation, users were divided into 4 groups, i.e. they were asked to dispose their contact lenses either after 1 day, 1 week, 2 weeks and 1 month of usage. These contact lenses were processed and analysed using scanning electron microscope to determine the presence of biofilm.

Results: There were 32 participants, majority (29) were females. Half were using low water, non-ionic polymer contact lenses while the rest were using high water, ionic polymer contact lenses. Biofilm was found in 50% (16) of the contact lenses. Among them, 62.5% (10) had biofilm formation on both the convex and the concave surfaces of the lenses. 25% (4) had biofilm on the concave surface only, while 12.5% (2) had biofilm on the convex surface

alone. The likelihood of biofilm formation on contact lenses increased with the duration that the lenses were used for.

Conclusion: Soft disposable contact lenses are known to be a significant causative factor for infective keratitis. This study showed that bacteria in the form of biofilm were present on contact lenses. Disposable contact lenses should be discarded within their recommended time as the risk of biofilm formation increases with usage.

II176/SL

The Intramuscular Nerve Branch in a Lacerated Skeletal Muscle Plays an Important Role in Muscle Recovery After Repair

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Aim: The recovery of lacerated skeletal muscles after repair never completes. Often the intramuscular (IM) nerve is cut and never repaired. This study investigates the recovery in a completely lacerated muscle repaired by epimysial suturing of the cut muscle ends. Two muscle groups were compared, 1 with the main IM nerve cut and the other with the IM nerve preserved intact.

Methods: The medial gastrocnemius (MG) of the adult NW White rabbit was used, with the contralateral muscle as the control. Under general anaesthesia, the laceration was done at the proximal quarter of the muscle, distal to the motor-point of the branch from the tibial nerve.

Results: The lacerated MG with its IM nerve preserved showed improved muscle wet weight, near normal morphology and contractile properties, with return of muscle fibre ratio and size at 28 weeks. The lacerated MG with its IM nerve cut demonstrated fibrosis, mononuclear proliferation with fatty infiltration, increased type I fibres and muscle atrophy of type II muscle fibres in the distal portion at 20 and 28 weeks, resulting in a poorer muscle recovery.

Conclusion: This study was motivated by the clinical question whether the IM nerve should also be re-anastomosed when repairing the lacerated muscle. Our hypothesis is that the IM nerve plays a key role in muscle repair and recovery. This study prompts the microsurgeon to repair the IM nerve in a lacerated muscle for improved recovery and return of function.

II177/SL

Tissue Engineered Vascularised Flaps with PLGA Scaffold: A Model for Soft Tissue Reconstruction

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Aim: The potential application of vascularised tissue engineered flaps is vast. This includes reconstruction of defects secondary to tumour resection, trauma, congenital abnormalities or burns. It addresses the difficult clinical problem of donor site morbidity encountered with harvesting flaps. This project aims to investigate the feasibility of developing tissue engineered vascularised flaps for reconstructive surgery.

Methods: A vascular supply was provided for using a ligated arteriovenous bundle model, as described by Tanaka et al. The arteriovenous bundle is placed into a poly-lactic-co-glycolic acid (PLGA) mesh seeded with human dermal fibroblasts. PLGA has been found to be a suitable dermal matrix, promoting homogenous cell distribution and tissue formation, based on in vitro studies. These experiments were carried out in male nude rats. The rats were sacrificed at the end of 4 weeks and the constructs were harvested. Histological and immunohistological studies were then carried out to evaluate the vascularisation of these constructs and the extent of tissue formation within the matrices.

Results: Histological studies demonstrate neovascularisation within the matrices and these arose from the vascular pedicle. Human fibroblast seeded on the matrices remained viable over the 4-week period of implantation as demonstrated by cell tracer studies. Neo tissue formation within the matrices was also seen on routine histology and on immunohistochemistry.

Conclusion: These results demonstrate that vascularised PLGA mesh seeded with human fibroblasts may serve as a viable model for constructing a vascularised flap.

II178/SL

Activated Caspase in Human Traumatic Brain InjuryJHY WONG¹, WL TAN¹, I NG²¹Department of Research, National Neuroscience Institute, Singapore,²Department of Neurosurgery, National Neuroscience Institute, Singapore

Aim: Apoptosis may play a role in cell death in head injury. The activation of caspase-3 has been considered the final common pathway for apoptotic cell death. Many animal studies have suggested that apoptosis occurs after TBI and the injury is directly related to the predominant localisation of cleaved caspase-3 in neurons. In this study, we sought to determine if the location of this activated apoptotic caspase in animal models directly reflect or illustrates the phenomenon in human pericontusional tissue.

Methods: Pericontusional tissues were collected from 14 patients during surgical treatment for intracranial hypertension. The tissues were fixed in 4% paraformaldehyde and sectioned using a cryostat and mounted on poly-L-lysine coated slides. The sections were double immunofluorescence labelled with cleaved Caspase-3 and NeuN or GFAP for neuronal or glial cells respectively.

Results: Cleaved caspase-3 was expressed in all 14 tissues collected and expression was found in neurons only. The glial cells did not show evidence of activated caspase-3 staining.

Conclusion: Our results suggest that although cleaved caspase-3 is expressed in neuronal and glial cells of animal TBI models, activated caspase-3 is found only in human neuronal cells. This may be an important consideration in the development of novel pharmaco-therapeutic strategies that specifically target apoptosis.

II179/SL

Establishing Human Islet Isolation Unit for Clinical Islet TransplantationDZM WANG¹, XJ WANG¹, KO LEE², RY CALNE¹, JR ISAAC¹¹Department of Surgery, Faculty of Medicine, National University of Singapore, Singapore, ²Department of Medicine, Faculty of Medicine, National University of Singapore, Singapore

Aim: The Edmonton Protocol has demonstrated that human pancreatic islet transplantation could lead to long-term (>12 months) insulin independence for Type 1 diabetes patients. NUS/NUH Department of Surgery is coordinating a clinical programme to replicate the Edmonton Protocol. The programme is to provide new medical technologies available in Singapore. The human islet isolation unit forms a major part of this programme, and it has been finally completed after more than 2 years of hard work.

Methods: The human islet isolation involves the following steps: pancreas procurement from cadaveric donor, controlled enzyme delivery via duct perfusion, pancreatic tissue enzymatic digestion, islet purification, islets counting, in vitro and in vivo functional evaluation of the islet, and preparing for islet transplantation.

Results: The basic function of the human islet isolation unit in NUH/NUS is to provide transplantable human islets in a cGMP standard laboratory for the clinical islet transplantation. The unit consists of (1) well trained staff members, (2) isolation facility, and (3) standard operational protocols (SOPs) and cGMP practices for the operations.

Conclusion: The isolation facility includes the following components, each of them essential for the functional laboratory that will produce transplantable islets from cadaveric donor pancreas: (a) class 10,000 clean room, (b) preparation room, (c) storage room, (d) bio-safety cabinets, (e) refrigerated Cobe 2991 cell processor, (f) tissue culture incubators, (g) centrifuges, (h) perfusion chambers, (i) Ricordi Chambers, (j) microscope and camera, and (k) reagents and consumables.

II180/SL

Free Fat Injection: Analysis of Adipocyte Survival and its Clinical ImplicationsWC ONG¹, DTW LEONG², TC LIM¹, DW HUTMACHER²¹Department of Surgery, National University Hospital, Singapore,²Department of Bioengineering, National University of Singapore, Singapore

Aim: Autologous free fat graft has gained popularity as a filler due to its easy availability and abundance. Increasingly, it is used for facial rejuvenation in

the outpatient setting. Nonetheless, the results have been unreliable. The main shortcoming is the partial absorption of the injected fat, which necessitates over correction and repeated injection. We believe that the key to successful use of fat as a filler is the maintenance of its viability during processing and reinjection. Survival of cells after trauma of injection through small cannulas needs to be addressed.

Methods: The present study aims to assess the viability of cells after trauma of injection through various cannula sizes. Adipose tissue obtained from manual lipoaspiration, was divided into 5 samples. Four samples were injected through cannulas of different sizes with 1 control. One mL of each sample was explanted for culture. The rest of the sample was processed to obtain processed lipoaspirate, which was then cultured. An initial cell count of each sample was performed. Assessment of cell vitality and growth was carried out on day 21 and day 28.

Results: Cell vitality assays using Alamar Blue and histological examination demonstrated adipocyte proliferation in all cannula sizes. No statistical difference was demonstrated in cell count and adipocytes viability.

Conclusion: On the basis of these quantitative data, we conclude that subjecting the adipose tissue to the trauma of the small sized cannulas does not affect the cell viability.

II181/SL

Viability of Human Adipose Tissue Processed Cell Population Obtained from Pump and Syringe LiposuctionTC LIM¹, D LEONG², FT CHEW², DW HUTMACHER²¹Department of Surgery, Faculty of Medicine, National University of Singapore, Singapore, ²National University of Singapore, Singapore

Aim: Correcting soft tissue defects by autologous fat grafting is routine in plastic surgery. Liposuction for autologous grafting is done with a hand-held syringe. The vacuum pump-assisted technique is not used on the understanding that cells harvested are not viable due to the harsher suction conditions. There is as yet no study to ascertain the viability and adipogenic potential of the pump lipoaspirated cells.

Methods: The metabolic profiles of cells processed from pump (pPLA) and syringe (sPLA) lipoaspirates were assessed. These cells were induced along the adipogenic lineage to compare their adipogenic potentials. Metabolic estimates were determined with AlamarBlue™ assay. The extent of adipogenic differentiation was measured using a digital photo counting software.

Results: Metabolic profiles of all (n = 8) were similar (pPLA cf. sPLA over 10 days). There were more cells in the uninduced culture cf. adipogenic induction culture. Cells under induction do not proliferate in comparison to cells cultured with normal growth media (more found at the end of 2 months). Results indicated comparable metabolic activity and adipogenic potential of cell populations.

Conclusion: Adipose tissue presents itself as a potential source of stem cells for tissue engineering. Using pump-assisted liposuction to harvest large volumes of adipose tissue with ease and efficiency for stem cell therapies and/or tissue augmentation would be advantageous and reduce operation time. This study is the first novel step towards the possibility of aspirating fat with a pump and was supported by grant number NMRC/0747/2003.

II182/SL

Cranioplasty Following Trephination Using a Novel Biodegradable Burr Hole CoverTC LIM¹, JT SCHANTZ¹, N CHOU², DW HUTMACHER³¹Department of Surgery, Faculty of Medicine, National University of Singapore, Singapore, ²Department of Surgery, National University Hospital, Singapore, ³National University of Singapore, Singapore

Aim: Rapid prototyping technologies have successfully been introduced in diagnosis, in surgical planning and in the fabrication of custom made implants for craniofacial reconstruction. A novel biodegradable polymers implant made of olycaprolactone by rapid prototyping technology fused deposition modelling and evaluated them in a clinical pilot study for cranioplasty.

Methods: Five patients with the diagnosis of chronic subdural haematoma were included in the study. Following trephination and evacuation of the

subdural haematoma, the burr hole (14 mm in diameter) was closed using a biodegradable polymer plug made of polycaprolactone (PCL). The implants have a computer controlled design with an upper rim diameter of 16 mm, a body of 14 mm in diameter, and fully interconnected honeycomb-like pore architecture of 400-600 micro metres in size.

Results: Postoperative CT scans indicated that the plugs were stable anchored in the osseous host environment and with no fluid collection present. The postoperative course was uneventful and patients were discharged after 5 days. Follow up scans after 3, 6 and 12 months showed the implants in situ, well integrated in the surrounding calvarial bone with new bone filling out the porous space.

Conclusion: These novel polymer scaffolds made of the slow degrading material polycaprolactone represent a suitable implant for closure of post trephination defects. The potential of rapid prototyping techniques to fabricate customised implants based on imaging information in combination with the above described biomaterial processing therefore offers a promising therapeutic potential for calvarial reconstruction.

II183/SL

Histological Morphology and Biocompatibility of Thin PCL Films as a Dural Substitute in Rabbits

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Aim: The possibility of the transmission of Creutzfeldt-Jakob disease and slow virus infection (HIV) by cadaveric dural implants made it necessary to find a synthetic, biocompatible and absorbable substitute for dural implants. Polycaprolactone (PCL) has been considered as a potential substrate for wide applications, such as drug delivery systems, tissue engineered skin and scaffolds for supporting fibroblasts and osteoblasts. A preliminary study using processed thin PCL films as a dural substitute in rats has been shown to be successful. The main aim of this study is to evaluate the histological morphology and biocompatibility of thin PCL films in vivo as a dural substitute in rabbits

Methods: Fifteen New Zealand white rabbits, weighing 3-4 kg, underwent craniotomy and durotomy in which 2 full thickness circular dural defects were created bilaterally equidistant from the sagittal sinus in the pericranium. One defect was implanted with thin PCL film while the contralateral defect was implanted with resected autologous dura to serve as a control. Seven and 8 rabbits were sacrificed 6 weeks and 12 weeks postoperatively respectively, with histological specimens taken from the dural defect sites.

Results: No seizures, infection or neurological deficit was noticed in the rabbits during the follow-up period. Histological assessment of the control (dura) and PCL implant was similar.

Conclusion: PCL demonstrates a favourable biologic response as a dural substitute in rabbit model. It is a promising biomaterial for dural replacement. We wish to acknowledge NMRC/0459/2000 and NMRC/0747/2003.

II184/SL

Development of a Biodegradable Polycaprolactone Membrane as a Dural Substitute

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Aim: A variety of bioabsorbable synthetic materials such as polyglactin and polydioxanone have been applied as dura substitutes but have proven to be less than satisfactory, causing cortical adhesions and persistent inflammatory reactions. In this study, the authors review their preliminary experience with an ultrathin biodegradable polycaprolactone (PCL) membrane.

Methods: In 12 New Zealand white rabbits, 2 full thickness circular bone flaps lateral to the sagittal sinus were excised and on the left side the dura was removed. The PCL membranes (Ø 10 mm) were treated with sodium hydroxide, and then implanted and sealed with fibrin glue. On the right side, the dura was excised and re-attached as an autograft. The dural grafts were removed after 6 and 12 weeks examining for cortical adhesions and a sample of cerebrospinal fluid was taken to examine for the presence of inflammatory immunogens. The surrounding bone and brain was also removed en bloc,

fixed in 2.5% formaldehyde and examined macroscopically and microscopically.

Results: No clinical complications were observed in the weeks following surgery. In all the animals, there were no cortical adhesions and a translucent membrane had regenerated over the PCL implant. No inflammatory immunogens were found in the cerebrospinal fluid. Histological examination revealed that the neomembrane consisted of fibroblasts and a regular network of collagen fibres.

Conclusion: This preliminary study shows that polycaprolactone can be a suitable dural substitute. However, long-term and clinical studies must be carried out to ascertain its use in humans and rule out the possibility of adverse effects.

II185/SL

Catheter Associated Urinary Tract Infection in a Tertiary Hospital and its Resistance Profile Over Time

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Aim: To identify the predominant uropathogens responsible for catheter associated urinary tract infection (CAUTI) and its antibiogram percentage resistance over time.

Methods: Comparison of urine culture results of catheterised patients in surgery wards in 1990 and 2003 was done. Positive urine culture infective organisms and antimicrobial resistance susceptibility data were collected.

Results: The most common organisms identified in 1990 and 2003 were *Escherichia coli*, *Klebsiella* sp., *Proteus* and *Acinetobacter*. *Pseudomonas* and methicillin-resistant *Staphylococcus Aureus* (MRSA) have emerged as new strains of catheter associated urinary tract infection. From 1990 to 2003, most antimicrobials have shown increasing resistance over time. Ciprofloxacin has shown the worst antibiotic resistance over time. Ampicillin, imipenem and co-trimoxazole have maintained their resistance to *Klebsiella* over time. Amikacin has shown good results of decreased resistance to *Klebsiella* and the same with cefuroxime to *Proteus* sp. Imipenem maintains to have no resistance to *E. coli*. There are 6 cases of MRSA with no resistant strains to drug of choice, vancomycin. In vitro culture studies also show no resistant strains to nitrofurantoin and fusidic acid. For pseudomonas, there are varying levels of resistance but ciprofloxacin has shown the highest resistance.

Conclusion: Most common infective organisms associated with CAUTI (*Escherichia coli*, *Klebsiella* sp., *Acinetobacter* and *Proteus* sp.) have not changed over time. However, there is emergence of *Pseudomonas* and MRSA as uropathogens of CAUTI. There is a general increase in resistance of infective organisms to majority of available antimicrobials. Strict antibiotic surveillance policies, asepsis, judicious use of antimicrobials and early catheter removal are recommended.

II186/SL

Periapical Dynamics: Possible Cause of Persistent Periapical Periodontitis

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Aim: Periapical diseases are dynamic in nature with numerous associate factors and interactions. An understanding of these factors and interactions is essential for clinicians during diagnosis and treatment. Previous studies have highlighted that percolation of periapical exudate into an incompletely filled root canal accounted for 60% of endodontic failures. However, these studies did not show how and why leakage of periapical exudate occurs and their role in persistent infection. This study aims to investigate the biomechanical response at the periapical region and correlate these findings with microscopic investigation of the apical portion of extracted endodontically treated teeth.

Methods: The experiments were conducted in 3 stages. (1) Digital photoelastic experiment was conducted to study the behaviour of dento-osseous structures during biting. (2) In vitro cyclic testing on extracted teeth was conducted to

study the fluid dynamics at the periapical region during biting. (3) Scanning Electron Microscopy examination of the root apex of extracted, previously endodontically treated teeth was conducted.

Results: These experiments showed distinct stress distribution and 'bite pressure influenced fluid movement' at the periapical region. This resulted in a significant influx of fluid from the periapical region into the apical portion of the root canal ($P < 0.001$). The microscopic examination of clinical specimens displayed multi-bacterial biofilm in the extraradicular and apical region of endodontically treated teeth.

Conclusion: It is apparent from this study that biting forces would result in periapical fluid influx, and this may facilitate extraradicular bacterial biofilm formation, and subsequent persistent apical periodontitis.

II187/SL

Effects of Acidic Dietary Liquids on Resin Restorative Materials

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Aim: To investigate the effects of pH on surface microhardness of resin-based restorative materials.

Methods: The materials evaluated included an ormocer (Admira [AM]), a giomer (Beautifil [BF]), 2 compomers (Compoglass F [CF], Dyract Extra [DY]), a minifill (Esthet X [EX]) and a nanofill (Filtek Supreme [FS]) composite. Eight specimens were made for each material-medium. Immediately after light polymerisation, the materials were preconditioned in Fusayama's artificial saliva (AS) at 37°C for 24 hours. They were then stored in citric acids with following pH values at 37°C for 1 week: pH 4, pH 3 and pH 2. Specimens stored in AS (pH 5.5) were used as control. After conditioning, Knoop Hardness Number (KHN) of the 6 materials was examined with a digital microhardness tester. The mean KHN was subsequently calculated and tabulated. Data were subjected to ANOVA/Scheffe's test ($P < 0.05$). The surface morphology was examined by Scanning Electron Microscope (SEM).

Results: With the exception of the ormocer AM, all materials were significantly softened after conditioning in citric acid with pH 3. Resin-based materials with glass-ionomer constituents (BF, CF and DY) showed dramatic decrease in hardness with decreasing pH values. SEM examination showed that the aluminosilicate glass fillers were susceptible to dissolution by acids.

Conclusion: Resin-based dental composites may be degraded by acids of low pH. Materials with glass-ionomer constituents were more susceptible to acid degradation. The effects of pH on change in surface hardness were material dependent.

II188/SL

Influence of Food Liquids on the 2-Body Wear of Resin Restorative Materials

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Aim: This study investigated the influence of food-simulating liquids on resin-based restorative materials.

Methods: The materials evaluated included an ormocer (Admira [AM]), a giomer (Beautifil [BF]), 2 compomers (Compoglass F [CF], Dyract Extra [DY]), a minifill (Esthet X [EX]) and a nanofill (Filtek Supreme [FS]) composite. Six specimens were made for each material-medium. After light polymerisation, the materials were preconditioned in Fusayama's artificial saliva (AS) at 37°C for 24 hours. The materials were then stored in the following food-simulating liquids at 37°C for 1 week: heptane (HP), 50% ethanol-water solution (ES), citric acid at pH3 (CR) and distilled water (DW). Specimens stored in AS were used as control. After conditioning, the materials were subjected to wear testing using a reciprocal compression-sliding wear instrumentation. Wear depth was measured using profilometry every 10,000 cycles up to 30,000 cycles

Results: Results of statistical analyses (ANOVA/Scheffe's; $P < 0.05$) of wear data were as follows: AM-AS, DW > CR, ES > HP; BF- ES, AS > CR, HP > DW; CF-AS > DW, CR, ES > HP; DY-AS, DW, ES > HP, CR; EX-DW > HP,

ES > AS, CR; and FS-AS > HP, DW, ES, CR (> indicates significantly more wear). With the exception of EX, the greatest wear was observed with conditioning and wear testing in AS. This ranged from 90.0 (5.5) μm for BF to 52.5 (3.7) μm for DY.

Conclusion: The effects of the different food-simulating liquids on 2-body wear were material-dependent.

II189/SL

Effect of Aqueous Environment on Surface Properties of Highly Viscous Glass Ionomers

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Aim: This study was conducted to evaluate the effect of aqueous environment on surface mechanical properties of highly viscous glass ionomer cements (HVGICs).

Methods: Three different HVGICs were selected for this study: Fuji IX Fast ([FN], GC), KetacMolar ([KM], ESPE) and KetacMolar Quick ([KQ], ESPE). Fourteen specimens were made for each material and kept in 100% humidity at 37°C for 1 hour. Specimens were then divided into 2 groups ($n = 7$): Group A – stored in 100% humidity at 37°C; Group B – stored in distilled water at 37°C. After 4 weeks, surface hardness and elastic modulus were determined by depth-sensing micro-indentation testing. Results were analysed using ANOVA/Scheffe's post-hoc test ($P < 0.05$).

Results: Mean displacements were from 24.2 to 28.4 μm that were greater than glass particle size in HVGICs. Mean hardness ranged from 65.2 to 98.2 HV while mean modulus ranged from 14.3 to 19.3 GPa. No significant differences were observed between HVGICs in Group A. Depending on the type of HVGICs, specimens in Group B showed increased or similar hardness and modulus when compared with those in Group A.

Conclusion: Regardless of the type of HVGICs, exposure to an aqueous environment may be beneficial for improving or maintaining surface mechanical properties of HVGICs.

II190/SL

Effect of Environmental pH on Surface Hardness and Indentation Modulus of Glass Ionomers

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Aim: This study was to evaluate the effect of environmental pH on surface hardness and modulus of resin-modified glass ionomer (RMGIC) and highly viscous glass ionomer cement (HVGIC).

Methods: A RMGIC (Fuji II LC, [FL], GC) and a HVGIC ([FN], GC) were evaluated in this study. Three solutions with constant concentration of KCl (150 mM) at different pH were used: Group A – pH 7; Group B – pH 5; Group C – pH 3. They represented pH of saliva, critical pH of demineralisation and pH of acidic beverage. Seven specimens of each material and solution were made and kept in different pH conditions at 37°C for 4 weeks. Surface hardness and elastic modulus were determined by depth-sensing micro-indentation testing. Results were analysed using ANOVA/Scheffe's post-hoc test ($P < 0.05$).

Results: HVGIC showed higher hardness/modulus than RMGIC in all groups. The variation in hardness and modulus with environmental pH were observed in both HVGIC and RMGIC: Hardness – Group A, Group B > Group C; Modulus – Group A > Group B > Group C.

Conclusion: Acidic beverages might have negative effects on surface properties GICs. The surface properties of RMGIC and HVGIC are not affected by the pH of saliva and the critical pH of demineralisation.

II191/SL

Early Expression Profile of PDGF in a Tooth Replantation Model

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Aim: To investigate PDGF expression, during the early healing phase of

immediate and delayed replanted teeth compared to the normal periodontium using immunohistochemistry.

Methods: Fifty-eight roots from 6 dogs were endodontically treated and extracted to simulate avulsion injury. Non-experimental group teeth were not extracted. In immediate replantation group, the teeth were replanted immediately while roots in the delayed group were bench-dried for 1 hour prior to replantation. The roots and surrounding structures were harvested after ½, 2, 3 and 4 days observation for immunohistomorphometric evaluation of the percentage of immunopositive cells and the immuno-intensity of the ECM of cementum, PDL and bone. Kruskal-Wallis and Mann-Whitney U tests were used for statistical analysis.

Results: PDGF was observed in the cells and ECM of all the 3 periodontal structures of the non-experimental group. The percentage bone cell count was significantly lower than cementum at ½ day ($P = 0.01$) and 3 days ($P = 0.02$) delayed replantation group; as well as lower than PDL at ½ day ($P = 0.01$) and 3 days ($P = 0.00$) immediate and ½ day ($P = 0.00$) delayed replantation groups. There was no significant difference among the non-experimental and experimental groups at any particular observation time.

Conclusion: PDGF was expressed in the 3 periodontal structures of the non-replanted and healing replanted teeth under optimal and adverse condition, implicating the role of PDGF in periodontal healing of replanted teeth. PDGF might up-regulate the paravascular progenitor cells during the healing process.

II192/SL

Effects of Ageing on Shear Strength of Dental Composite Restoratives

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Aim: This study aimed to investigate the effect of ageing on the shear-punch strength of dental composite restoratives.

Methods: The materials investigated were from the same manufacturer (3M ESPE) and included Z100, Filtek Z250, Filtek Flow (FF), F2000 and A110 composites. Fourteen specimens (8.0 mm diameter and 1.0 mm thick) of each material were prepared, randomly divided into 2 groups of 7 and conditioned for 1 week and 1 month, respectively, at 37°C. At the end of the conditioning period, the specimens were restrained and subjected to shear-punch strength testing using a 2.0 mm diameter punch at a crosshead speed of 0.5 mm/min. The shear punch strength (sS) of the specimens was computed and data was subjected to ANOVA/post-hoc Scheffe's tests and independent samples *t*-test at a significance level of 0.05.

Results: At 1 week, the sS of Z100 was significantly higher than all other materials evaluated. The resistance to shear deformation of A110 and Z250 was significantly higher than F2000, which was higher than FF. Same material ranking was observed for the 1 month specimens with the exception that there was no significant difference between Z100 and Z250. The sS of FF was significantly higher after 1 month of aging.

Conclusion: The effect of ageing on shear strength of resin-based dental composite restoratives was material-dependent. The shear strength of microfilm FF, which was GDMA based, was significantly higher after ageing.

II193/SL

Mechanical Characterisation of New Monomers Synthesised for Dental Restorations

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Aim: The objective of this study was to determine and compare the hardness and modulus of newly synthesised POSS based dental polymers (MA-POSS and ME-POSS) with unfilled 1:1 Bis-GMA/TEGDMA polymeric resins (control). The setting reaction of all the polymeric resins in air and distilled water stored at 37°C was also investigated over a 7-day period.

Methods: The hardness and modulus of the materials ($n = 8$) were determined using depth-sensing microindentation testing with the Instron Micro Tester at time intervals of 0, 1 and 7 days. Hardness data was obtained by dividing

the peak load over the maximum projected contact area while modulus was calculated by analysis of the loading/unloading load-displacement (*P-h*) curves and the analytical model according to Oliver and Pharr. Results obtained were analysed using 1-way ANOVA/Scheffe's post hoc test ($P < 0.05$).

Results: When stored in both air and distilled water, both hardness and modulus of MA-POSS and ME-POSS were found to be significantly lower than the control at all time intervals. Elastic modulus and hardness generally increased on storage. Both hardness and modulus decreased on day 7 after storage with the exception of hardness values for ME-POSS.

Conclusion: POSS-based dental polymers resulted in lower hardness and modulus values when compared to the conventional Bis-GMA/TEGDMA systems. All polymeric resins continued to polymerise for at least 1 day after light activation.

II194/SL

Osteogenic Differentiation of Mesenchymal Stem Cells from Bone Marrow

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Aim: Mesenchymal stem cells (MSCs) represent an ideal source for cell therapy especially for bone regeneration.

Methods: In this study, iliac marrow was extracted and MSCs were isolated. Due to limited number of true MSCs in bone marrow, MSCs were expanded to a large number in vitro for 14 days. MSCs were then induced to differentiate in the DMEM medium with 10% FBS supplemented with β -glycerol phosphate, L-ascorbic acid and dexamethasone. The medium was changed every 3 days.

Results: After 21 days, differentiated MSCs were characterised as osteogenic cells in HE, Von Kossa, Alizarin red stained histology, ALP histochemistry, immunohistochemical staining against osteonectin (ON) and osteopontin (OP) and quantitative measurements of ALP activity.

Conclusion: Bone marrow MSCs can be differentiated into osteogenic cells and this might be a potential therapy for various applications.

II195/SL

Directed Differentiation of Adult Bone Marrow Mesenchymal Stem Cells Towards Cardiomyocytes

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Aim: Transdifferentiation of adult bone marrow stem cells (ABMSCs) into cardiomyocytes is a rare phenomenon and thus remains a poorly understood mechanism. We seek to characterise the cardiomyogenic plasticity of ABMSCs under the influence of 3 classes of differentiating agents.

Methods: Bone marrow cells were isolated from the sternum of patients undergoing coronary artery bypass graft surgery. Plastic-adherent mesenchymal stem cells (MSCs) were obtained from marrow cells following depletion of mature blood cells and purification in a Histopaque-Ficoll gradient. The MSCs were then separately cultured in DMEM or a cardiomyocyte-promoting medium (CM) supplemented with 5-azacytidine (CM-AZA), butyric acid (CM-BA) or amphotericin-B (CM-AMB). The expression of specific cardiomyogenic markers was assessed by RT-PCR on mRNAs extracted from MSCs cultured under different conditions.

Results: CM promoted the expression of cardiac α -actin (CAA), myocyte enhancer factors (MEFs) 2A, 2C and 2D, troponin-c, troponin-t and skeletal muscle α -actin (SMAA) in the differentiating cells. Only basal expression levels of CAA, MEFs 2A, 2C and 2D were detected in cells cultured in DMEM. Cells treated with AZA, BA and AMB showed similar expression profiles of cardiac markers, although cardiac troponin-t expression was notably induced with BA and AMB.

Conclusion: Human adult bone marrow stem cells can be differentiated in CM to become cardiomyocytes. These ex-vivo differentiated cardiomyocytes may be potentially valuable for repairing damaged myocardium.