

Modern Medicine: To Share, To Educate, To Investigate

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2003 is a year no Singapore doctor or healthcare professional will ever forget.

In the first ever analysis of the world's healthcare systems, the World Health Organisation's World Health Report¹ ranked Singapore 6th among 191 health systems it had evaluated. Singapore was the only Asian country other than Japan to be ranked amongst the top 10 countries, and was ahead of both the United Kingdom and the United States of America. Our handling of the Severe Acute Respiratory Syndrome (SARS) crisis gave further credence to these results, and demonstrated to all that ours is a world-class healthcare system comprising trained and motivated doctors, nurses, administrators and allied health professionals.

Our small country has attained global stature in healthcare by continually investing in talent development. Until now, this meant identifying and grooming talent locally, sending staff overseas for further learning and skills development, and encouraging participation in continuing medical education programmes. In this new biotech century, this will not be enough to stay ahead: we need also to engender a climate of collaboration and research, a pivotal strategy for our survival in the increasingly competitive knowledge economy.

The National Healthcare Group (NHG) Annual Scientific Congress is a unique experience in that it is not a specialised scientific meeting, nor is it a 'doctors only' meeting, but a true *healthcare conference* aiming to integrate all aspects of healthcare delivery that ultimately reaches the individual patient. This is in keeping with the NHG motto of "patients first". With increasing participation from the region, it is likely that this congress will soon become the leading *healthcare conference* in Asia.

This year's Congress focuses on three activities: *to share, to educate, to investigate*. SARS made us realise that it is a small world after all, and that the best way for us to battle new diseases is to efficiently share information with healthcare workers globally. Some scientists have described SARS as only the dress rehearsal.² Thus, the SARS symposium represents not only a platform for us to share information on this disease today, but also the development of a forum for exchange of knowledge on future rapidly emerging illnesses. Multidisciplinary educational symposia that have been organised over the two conference days will update participants on the latest developments and newest ideas within their fields. Most importantly, research within NHG will be showcased in almost 400 poster and oral presentations. Together, this integrated approach of sharing the knowledge it acquires, continually educating and updating its healthcare workers, and encouraging a research climate to push the frontiers of medical science will secure Singapore's place as a leading healthcare hub, regionally and globally.

This year, the Congress has taken yet another bold step towards international recognition. For the first time in Singapore's medical history, abstracts and proceedings from a local medical conference will be immortalised in a peer-reviewed, indexed medical journal. The Congress Proceedings is published in this supplement issue of the *Annals, Academy of Medicine, Singapore*. All abstracts were peer-reviewed, and of the 481 abstracts received 89 (18.5%) were rejected, a rate similar to top international conferences. Based upon merit, 33 participants were invited to submit extended abstracts. Of these, 20 were selected to compete for the Young Investigator Award and Best Oral Presentation Awards, and the remainder will be presented as Special Posters. This rigorous peer-review system has ensured that only high-quality scientific content will be presented at the Congress, and that every oral presentation will be of international standing.

Despite difficult times, the Congress has proceeded as planned. This would not have been possible without the excellent efforts of all Singapore healthcare workers in battling SARS, the faith and perseverance of the entire organising committee, the enthusiastic participation and timely submissions by over 500 educators and investigators, the support of the senior management of NHG, and you the participants. The Organising Committee hopes you enjoy reading these congress proceedings as much as we have enjoyed putting them together for you, and most of all that you have an enlightening and rewarding conference.

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Programme at a Glance

Day 1 (4th October 2003, Saturday)

Time	Collyer	Sophia	Olivia	Morrison	Moor	Orchard	Minto	VIP A	Bras Basah A	Bras Basah B
7.15am to 8.15am	Bayer Morning Symposium (Canning)									
8.30am to 10am	SARS When the Wrong Sun Shines – Clinical Features & Pathology			NEUROLOGY Migraine – Modern Ailment: Looking for a Solution	PATHOLOGY/ LAB MED Modern Trends in the Laboratory Diagnosis for Physicians	GERIATRICS PreventiveGeriatrics – Body, Mind and Hormones	GENERAL SURGERY Minimally Invasive Modern Surgery	RHEUMATOLOGY ALLERGY & IMMUNOLOGY Drug Allergy Update	FREE PAPERS	FREE PAPERS
10am to 10.30am	TEA BREAK									
10.30am to 12.30pm	OPENING CEREMONY CUM PLENARY LECTURE (STAMFORD BALLROOM)									
12.30pm to 1.30pm	ELI-LILLY LUNCH SYMPOSIUM (Padang) LUNCH POSTER JUDGING (Mercury, Enterprise & Hullet)									
1.30pm to 3.00pm	SARS Playing Cat & Mouse – Aetiology & Epidemiology	NURSING Modern Medicine for Modern Ailments – Nursing Perspectives I	UROLOGY Update on Treatment for Modern Urology Ailments	FAMILY MEDICINE Preventive Health – Redefining the GP's Role I	PATHOLOGY/ LABMED Modern Trends in the Laboratory Diagnosis of Lymphoma and itsClinical Significance	OBSTETRICS GYNAECOLOGY & NEONATOLOGY Modern Technology	MENTAL HEALTH Abuse and Addition to Prescription Drugs – New Implications for Medical Practice I	CLINICAL PRACTICE IMPROVEMENT PROGRAMME (CPIP) Convention I	FREE PAPERS Young Investigator's Award	
3.00pm to 3.30pm	TEA BREAK									
3.30pm to 5.00pm	SARS Taming The Shrew – Pathogenesis & Therapeutics			FAMILY MEDICINE Preventive Health – Redefining the GP's Role II	PAEDIATRICS Paediatrics for General Practitioners	OBSTETRICS, GYNAECOLOGY & NEONATOLOGY Screening	MENTAL HEALTH Abuse and Addition to Prescription Drugs – New Implications for Medical Practice II	CLINICAL PRACTICE IMPROVEMENT PROGRAMME (CPIP) Convention II	GASTROENTEROLOGY “All That Trouble and My Scope is Normal?” –Management Options in Functional Gastrointestinal Disease	
7pm	CONGRESS DINNER AND 2ND NHG LECTURE – By Invitation Only (STAMFORD BALLROOM)									

* Mercury, Enterprise and Hullet will be used for poster display
The Secretariat Office and Speakers Preparation Room are situated in Wilberforce

Day 2 (5th October 2003, Sunday)

Time	Sophia	Olivia	Morrison	Moor	Hullett	Orchard	Minto	VIP A	VIP B	Bras Basah A	Bras Basah B
7.15am to 8.15am	STRYKER MORNING SYMPOSIUM (Collyer)										
8.30am to 10am		CHAIRMAN'S SYMPOSIUM: LIFE SCIENCES: Cancer, Chromosomes, Genes & Stem Cell Therapy I	ALLIED HEALTH Diabetes, Head to Toe: A Multidisciplinary Management Approach	NEUROLOGY Multidisciplinary Approach of Sleep Disorders	ANAESTHESIOLOGY Advances in Acute and Chronic Pain I	DIAGNOSTIC RADIOLOGY Musculoskeletal Radiology: Beyond the Bare Bones	DENTISTRY Life Sciences Developments in Dentistry I	EVIDENCE-BASED NURSING Workshop I	TRAUMA Who Wants to Manage Trauma? – The 2nd Edition I	ORTHOPAEDICS Bringing Out the Orthoped in you – A Hands-On Bioskills Workshop for the General Practitioner on Common Orthopaedic Surgical Procedures Workshop I	OTORHINOLARYNGOLOGY Advances in Otorhinolaryngology
10am to 10.30am	TEA BREAK										
10.30am to 12pm	SARS Behind Bars – Infection Control	CHAIRMAN'S SYMPOSIUM: LIFE SCIENCES: Cancer, Chromosomes, Genes & Stem Cell Therapy II	ALLIED HEALTH Occupational Stress and Strain – Do We Have to Live With It?	INTERNAL MEDICINE Recent Advances in Internal Medicine: Multidisciplinary Snapshots	ANAESTHESIOLOGY Advances in Acute and Chronic Pain II	DIAGNOSTIC RADIOLOGY Advances in Diagnostic Imaging	DENTISTRY Life Sciences Development in Dentistry II	EVIDENCE-BASED NURSING Workshop II	TRAUMA Who Wants to Manage Trauma? – The 2nd Edition II	ORTHOPAEDICS Bringing out the Orthoped in You – A Hands-On Bioskills Workshop for the General Practitioner on Common Orthopaedic Surgical Procedures – Workshop II	ENDOCRINOLOGY Thyroid Cancer Update
12pm to 1pm	PFIZER LUNCH SYMPOSIUM (Padang) LUNCH										
1pm to 2.30pm	SARS General Practitioners' Symposium on SARS	DERMATOLOGY Pigmentation Symposium	CARDIOLOGY Modern Therapies for Heart Failure I	ORTHOPAEDICS Arthritis Symposium I – Managing Patients' Expectations in the Treatment of Degenerative Arthritis	NURSING Modern Medicine for Modern Ailments – Nursing Perspectives II	OPHTHALMOLOGY Angle Closure Glaucoma (ACG) in Singapore – The Eye Under Pressure I	EMERGENCY MEDICINE Disaster Preparedness: 2003 and Beyond I		GENERAL MEDICINE A Multidisciplinary Approach to Obesity Management I	MEDICAL ETHICS Clinical Ethics – Sense and Sensibility: Common Issues in Clinical Ethics	REHABILITATION MEDICINE Pain in the Neck and Arm - Something Old, Something New, Something Unusual – Workshop I
2.30pm to 3pm	TEA BREAK										
3pm to 4.30pm	SARS Mad About You - Psychosocial Issues	DERMATOLOGY Occupational Dermatitis Symposium	CARDIOLOGY Modern Therapies for Heart Failure II	ORTHOPAEDICS Arthritis Symposium II Current Concepts in the Operative Treatment of Degenerative Arthritis	DIGESTIVE SURGERY	OPHTHALMOLOGY Angle Closure Glaucoma (ACG) in Singapore – The Eye Under Pressure II	EMERGENCY MEDICINE Disaster Preparedness: 2003 and Beyond II		GENERAL MEDICINE A Multidisciplinary Approach to Obesity Management II	MEDICAL ETHICS Research Ethics – Ethical and Responsible Biomedical Research: New Challenges in a Modern Era	REHABILITATION MEDICINE Pain in the Neck and Arm – Something Old, Something New, Something Unusual – Workshop II

* Mercury and Enterprise will be used for poster display
The Secretariat Office and Speakers Preparation Room are situated in Wilberforce

SARS in Singapore: Looking Back, Looking Forward

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Introduction

Severe Acute Respiratory Syndrome (SARS) is a new infectious disease caused by a novel coronavirus. In Singapore, an outbreak of SARS was started by a traveler (Patient A) who had stayed at a hotel in Hong Kong, on the same floor as a doctor from southern China who was the index case for the large outbreaks in Canada, Vietnam and Hong Kong.^{1,2} Patient A returned to Singapore on 25 February and developed symptoms the same day. Patient A was subsequently admitted to Tan Tock Seng Hospital (TTSH) on 1 March 2003, was isolated on 6 March 2003 and was only recognised as a SARS case on 14 March 2003 after the World Health Organisation (WHO) raised a global alert.

The SARS outbreak in Singapore lasted for 11 weeks. There were a total of 238 probable cases of which 41% were healthcare workers, 37% were family, friends or visitors, and 13% were inpatients. A total of 205 cases recovered from SARS and 33 (14%) have died. The last SARS case had onset on 5 May 2003 and was isolated on 11 May 2003.

SARS in Singapore: Looking Back

The SARS outbreak was an unprecedented event in the history of Singapore medicine. In March 2003, very little was known about the new infection – how infectious it was, the spectrum of clinical presentations, the causative agent and the measures that were effective in protecting healthcare workers and stopping the spread of the infection. Many of the containment measures used in Singapore were applied empirically and were rapidly modified as new knowledge emerged.

Looking back, there are 3 key lessons which we learnt about the infectiousness and transmission of SARS that were critical in shaping our public health approach.

Firstly, it was recognised early on that SARS is predominantly a nosocomial infection. Out of the total of 238 SARS cases, there were 8 imported cases. Of the remaining 230 cases, 180 (78.3 %) were acquired in the hospital and nursing home setting. It is unclear why this is so. A key reason could be the fact that virus excretion tends to occur relatively late in the course of symptomatic disease, at which time patients are most likely to be in hospital. In addition, the cohorting of ill patients in general wards and the frequency and complexity of patient-patient and patient-staff interactions and movements are important contributory factors. Regardless of the reasons, the significance is that rapid and effective containment of hospital transmission is the key to control of a SARS outbreak.

A second key lesson is that infected patients can manifest

with a broad spectrum of presentations. The SARS case definition required the presence of pneumonia or respiratory distress syndrome and a relevant history of travel or contact. We learnt, at great cost, that patients who are immunosuppressed or have chronic co-morbid conditions may have very atypical presentations of SARS infection. Such patients may have intermittent low-grade fever and may only show features of pneumonia very late in the course of infection. Some patients may even have concurrent bacterial infections that very effectively mask the presence of SARS infection. In recognition of atypical presentations of SARS, we had to modify our detection and containment strategies markedly to minimise the risk of transmission through such cases.

Thirdly, we learnt that most SARS patients are in fact not very infectious. The majority pass on the infection to zero, one or two cases. However, there are a small number of patients who are highly infectious, the so-called “super-spreaders”. Such patients typically infect more than 20 patients each directly. Unfortunately, in the Singapore outbreak, we had 5 “super-spreaders”, 3 of whom occurred within the first 2 weeks of the outbreak. Again, it is not clear why some patients are “super-spreaders”. However, as it is not possible to identify which patients will turn out to be “super-spreaders”, the public health implication is that isolation and quarantine measures have to be cast as widely as possible so as to capture as high a percentage of SARS cases as early as possible.

SARS in Singapore: Looking Forward

Although SARS has been contained globally, the consensus among experts is that there is a serious possibility of a resurgence in the northern hemisphere winter. It is vital therefore that we enhance our level of preparedness so as to minimise the risk of a recurrent outbreak in Singapore.

It is critical for us to carry out broad-based surveillance to detect new or imported cases of SARS early. In setting up such a system, we need to strike a balance between the perceived risk and the cost of running a complex surveillance machinery. In Singapore, surveillance for SARS covers patients with atypical pneumonia who present at our hospitals, in particular those with a history of travel to a previously SARS-affected area in the preceding 10 days, and those who have not responded after 72 hours of antibiotic treatment. The surveillance system also covers patients with prolonged unexplained fever and a history of travel to a previously SARS-affected area in the preceding 10 days.

Temperature screening of inbound persons at our airports, seaports and land check-points is continuing. However, it is

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still possible that new imported cases may be missed. As SARS is predominantly transmitted in the hospital setting, it is therefore critical that stringent measures are in place to try to prevent the seeding of infectious SARS cases within unprotected areas in hospitals. As such, we have maintained the requirement for triaging of patients at Emergency Departments and Specialist Outpatient Clinics. This allows for febrile patients to be managed separately from non-febrile ones, and to be isolated if they are admitted. Healthcare workers in high-risk areas in hospitals and nursing homes are still required to wear N95 masks and other personal protective equipment to reduce the risk of transmission occurring through this group.

Despite these measures, there is still a risk that an externally introduced SARS case may lead to transmission of infection in the hospital. This is because of the non-specific nature of SARS and the absence of a sensitive diagnostic test which can pick up cases early in the course of illness. In view of this, it is essential that hospitals are able to pick up a new cluster of infections early. To achieve this, each hospital needs to have an effective system of temperature monitoring of all its healthcare workers so that it can be alerted to a situation where more than 3 healthcare workers and/or patients in the same clinical area become ill.

A key lesson that we learnt in managing SARS was that early detection and rapid response are absolutely crucial in containment of hospital outbreaks. For hospitals, the critical element of the rapid response component is the ability to quickly generate a complete list of all patients, healthcare and other workers, and visitors who have been in the affected clinical area during the time when active transmission was thought to be occurring. This is not an easy task given the numbers of individuals who pass through every clinical area every day and the complexity of human interactions and movements. Nevertheless, rapid and complete contact tracing is absolutely essential. In view of this, the Ministry of Health has been carrying out exercises with the hospitals to ensure that all of them have the necessary systems and people to respond effectively should the need arise.

If a hospital outbreak is not rapidly contained, there is an ever-present risk that transmission of infection may spill into the community. It is also possible that mutations in the SARS coronavirus may change the pattern of transmission. As such, it is critical that community contact tracing and quarantining of exposed persons can be carried out rapidly and effectively if there is a need to do so. To meet this need, the Ministry of Health has augmented its response capability and capacity by expanding its Operations Group and developing procedures to leverage on other agencies if there is a major surge requirement.

In Singapore and around the world, research on SARS is a major priority. This is vital as the new information emerging from these studies will help us be better prepared in the most cost-effective way. At the same time, it is envisaged that improvements in diagnostic tests and treatments will make the management of SARS cases simpler and produce better clinical outcomes. It is also very important that significant new findings are conveyed to the medical community and to the public in a timely way. This will allow medical professionals to be better equipped to assess patients and to identify and manage suspected SARS cases. It is also hoped that better understanding of SARS may help ameliorate the tremendous public fear which characterised the earlier part of the outbreak. This would help make the work of containment of new SARS clusters easier.

Acknowledgement

I would like to acknowledge the outstanding dedication and enormous efforts of all healthcare workers and hospital staff in Singapore which have contributed greatly to the containment of SARS.

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RUNX and Cancer

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There is a renewed realisation that dysregulation of cell differentiation induces cancer. This notion is partly based on the fact that many of the oncogenes and tumour suppressor genes so far identified are developmental regulators. The runt-related (RUNX) genes are the mammalian homologs of the *Drosophila* segmentation gene, runt.

There are three RUNX genes, RUNX1/AML1, RUNX2/CBFA1 and RUNX3/PEBP2aC, all of which have been shown to have oncogenic potential. The RUNX genes are now known to be involved in the regulation of diverse biological activities. RUNX1 is essential for the generation of hematopoietic stem cells and is most frequently involved in human acute leukaemia (30%), mainly as a target of chromosome translocations. RUNX2 is required for maturation of osteoblast. In the mouse system, the gene is shown to cooperate with c-myc to induce B-cell lymphoma. RUNX3 is most recently characterised and shown to be a tumour suppressor of gastric cancer. In this article, we will first describe how we are exploiting RUNX1 to hunt a large number of cancer causing genes and then describe our results on the involvement of RUNX3 in gastric cancer.

The past several decades of research have uncovered a number of oncogenes and tumour suppressor genes but the current list of these genes by itself does not explain how these genes are involved in multi-step carcinogenesis. On the one hand as we still continue searching for as yet unknown key cancer-causing genes we must, at the same time, devise an experimental system to analyse the relationship among these genes. These studies are important not only for the better understanding of carcinogenesis but also for creating a foundation for future drug discoveries. A powerful approach has been described for this purpose based on a mouse leukaemia model using retroviruses. This method relies on the property of a strain of retrovirus which integrates host cell DNA randomly and either activates oncogenes or destroys tumour suppressor genes to cause leukaemia. The best example is the BXH2 leukaemia-prone mouse. The BXH2 mouse contains an endogenous retrovirus which is released in the body and integrates randomly into host chromosomes. As a result of this, the mouse develops acute myeloid leukaemia at 10 to 12 months of age in 90% of all cases.

The viral integrations can be used as tags to easily fish out cancer associated genes by the inverse polymerase chain reaction (IPCR). Typically, three genes are affected by the retrovirus in this mouse model. Therefore, the BXH2

leukaemia-prone mouse strain is a high throughput method to detect cancer causing genes.¹ Although many (~50~100) potential cancer-causing genes have been identified by this method, functional interactions among the genes are not known. Therefore, putting all these genes in perspective to understand the grand view of carcinogenic processes is the subject of the next generation of research.

RUNX1 is the most frequent target of chromosome translocations in acute leukaemia. We found that haploinsufficiency of RUNX1 is also leukemogenic.² In fact, familial cases are known in which one allele of RUNX1 is mutated and family members frequently develop acute myeloid leukaemia (familial platelet disorder with propensity to acute myeloid leukaemia).³ Based on these observations, we attempted to generate a mouse model of FPD-AML and knocked out one of the alleles of Runx1 of BXH2 mouse to observe the leukemogenic process. In other words, we introduced one genetic change at the beginning of the experiment. As expected, we observed the development of leukaemia in BXH2 (Runx1+/-) mice two months earlier than the parental BXH2 mice, suggesting strongly that haploinsufficiency of Runx1 is leukemogenic also in the mouse and that the second and third alterations of genes which must have occurred in these mice cooperate with Runx1 (+/-). We expect that by identifying the altered genes, we will accumulate the genes which functionally cooperate with inactivated Runx1. The results will be critical in understanding the functional cooperation among cancer causing genes. Since RUNX1 is responsible for approximately 30% of all types of human leukaemia, the genes altered in Runx1+/- BXH2 (Runx1+/-) should be very valuable in the study of human leukemogenesis.

As multiple genetic changes, at least three in leukaemia, are required for the development of cancer, abnormality of only Runx1 is not sufficient to give rise to clinically overt leukaemia. If one of these additional hits is controlled adequately by a small molecule, there is a possibility that we will see a remission of the cancer in leukaemia patients.

We already have approximately 65 DNA samples from BXH2 leukaemias. Each leukaemia sample in BXH2 (Runx1+/-) usually contains three to five genetic hits (three to five retrovirus integrations in BXH2 mice).

The molecular mechanisms by which epithelial cells of gastric mucosa are converted to cancer cells are poorly understood. One of the main reasons is that the genes involved in normal development and differentiation of stomach epithelial

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cells, and those involved in the carcinogenic processes of cells are largely unknown.

We recently reported that the loss of expression of the runt-related gene 3 (RUNX3) is causally related to gastric cancer.⁴ RUNX3 is a member of the three gene family encoding the DNA binding subunit of the Runt domain transcription factor, PEBP2/CBF.⁵ In the mouse, Runx3 is ubiquitously expressed. In the stomach of the adult mouse, Runx3 is expressed most prominently in pepsinogen-producing chief cells and surface epithelial cells, both of which are terminally differentiated in gastric mucosa.

In Runx3^{-/-} mice, hyperplasia of gastric mucosa is observed due to insensitivity of the epithelial cells to TGF- β mediated growth inhibition and TGF- β induced apoptosis.⁴ 15 cell lines isolated from human gastric cancer⁷ showed no or reduced expression of RUNX3 due to hemizygous deletion and hypermethylation of the promoter region. In 46 surgically resected gastric cancer specimens, 9 out of 22 stage I (41%), 1 out of 2 stage II (50%), 11 out of 14 stage III (79%) and 7 out of 8 stage IV (88%) cases exhibited reduction of RUNX3 expression, respectively. Then we looked for mutations in RUNX3 in gastric cancer specimens and found one, R122C, in which arginine 122 was replaced by cystine after screening of more than 150 specimens. The mutation was located within the conserved Runt domain required for the DNA binding and heterodimerization with the β subunit.⁴

To test whether RUNX3 has a tumour suppressor activity, a cell line, MKN28 which does not express RUNX3, was inoculated subcutaneously in nude mice and found to be tumourigenic. Two independent MKN28 cell lines stably expressing exogenous RUNX3 were poorly tumourigenic. When two independent stable lines expressing the RUNX3 (R122C) were inoculated, no tumour suppressor activity was detected. These results strongly suggested that RUNX3 is a tumour suppressor of gastric cancer.

It is known that the silenced genes due to hypermethylation of the promoter region could be reactivated by treating cells with inhibitors of methyltransferase such as 5'-aza-cytidine and histone deacetylase, such as trichostatin A.⁶ We treated three gastric cancer cell lines which do not express RUNX3 with these inhibitors alone or in combinations and found that RUNX3 was indeed reactivated by either one of these inhibitors.

The results confirmed that the gene silencing is due to DNA methylation of the promoter region and recruitment of histone deacetylase near the promoter region.

As mentioned above, mutations in RUNX3 are very rare. Therefore, when the gene is reactivated in gastric cancer cells, the gene is, in most of the cases, free of any mutation. Therefore, there is a high hope that tumour growth in gastric cancer may potentially be controlled by inducing the expression of silenced RUNX3 using reagents such as histone deacetylase inhibitors. The mouse model of gastric cancer to test this possibility is being established.⁷

RUNX3 is on 1p36 where it has been predicted that an important tumour suppressor is located, since LOH of this locus is observed at high frequency not only in gastric cancer but also in many gastrointestinal and other cancers. RUNX3 may be this long sought-after tumour suppressor and, at the same time, the product of the gene may be the primary target of TGF- β -mediated tumour suppressor pathway. If these predictions are correct, RUNX3 would be involved in many different types of cancers.⁸

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Intrauterine Stem Cell Therapy

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Stem cells are found in embryonic, fetal and most adult tissues, and are characterised by two properties: the ability to self renew and to differentiate into multiple discrete tissue types. They offer great promise for tissue regeneration, cell replacement and gene therapy against a range of human diseases, but, with the exception of haemopoietic stem cells, clinical application at present remains largely speculative. Recent success with stem cell transplantation in adults and children, together with advances in stem cell biology and gene manipulation now render intrauterine correction of genetic defects using stem cells a realistic prospect.

Rationale

Genetic diseases with intrauterine onset can lead to intrauterine death or permanent neurological or musculoskeletal damage in early life. Examples include skeletal dysplasias, mucopolysaccharidoses, cerebral gangliosidoses, the leukodystrophies, a thalassaemia and muscular dystrophies. To prevent this, stem cells have been proposed as vehicles for fetal gene or protein delivery, because of their ability to expand and give rise to large numbers of multipotent cells. Transplantation in early intrauterine life has a number of advantages over postnatal transplantation, raising the possibility that intrauterine therapy may eventually be advantageous for genetic diseases of postnatal onset. Firstly, *in utero* therapy may be delivered in time to prevent irreversible disease manifestation (as in a thalassaemia or congenital myotonic dystrophy). Second, it obviates the severe treatment-associated morbidity associated with postnatal bone marrow transplantation. Third, the fetal environment is highly proliferative with exponential expansion of stem cell populations, and large-scale migrations of stem cells occur naturally only in fetal life. Next, there is a huge stoichiometric advantage in that the fetus is only about 75 g in size at 13 weeks gestation, allowing transplantation of many more cells than can be given postnatally. There is also some evidence that fetal cells have a competitive advantage over adult cells.¹ Finally, the immunological naiveté of the early gestational fetus produces tolerance to foreign antigens if presented before a critical window, around 12 to 14 weeks gestation.

The alternative approach is *in utero* gene therapy. For *in vivo* gene therapy, a vector containing the therapeutic gene is transferred directly to the fetus and transduces stem and other

cells *in situ*. The risk of unintentional transduction of germ cell or maternal cells to date has precluded work on human fetuses. Direct injection of gene vectors *in utero* has until recently proved disappointing in animal models, because of poor uptake of vectors in differentiated tissues, immune responses and transient transgene expression.² In contrast, *ex vivo* gene therapy uses autologous stem cells that are first obtained from the fetus, transduced *in vitro* and then transplanted back to the fetus, advantages being the selective transduction of target cells and minimisation of the immune response. Results from postnatal gene therapy trials validate the clinical effectiveness of the *ex vivo* approach.

Haemopoietic Stem Cell Transplantation

To date, intrauterine transplantation has mainly been evaluated using haemopoietic stem cells because of their well-established proliferative and multi-lineage potential and years of clinical experience in adult transplantation. Most attempts at haemopoietic stem cell transplantation *in utero* have failed, because the mid trimester fetus is immunocompetent and rejects allogeneic cells.³ Nevertheless, there are several well documented reports of successful engraftment and immune reconstitution following intrauterine transplantation of haploidentical paternal bone marrow in severe combined immunodeficiency.^{4,5} This disease, however, can instead be treated postnatally, either by allogeneic haemopoietic stem cell transplantation or by *ex vivo* gene therapy using autologous haemopoietic stem cells. Animal studies of intrauterine gene delivery using *ex vivo* transduced autologous haemopoietic stem cells have found limited transduction and sub-therapeutic expression with retroviral vectors, due to quiescence of haemopoietic stem cells and their low levels of the amphotropic receptor required for viral entry.^{3,6}

Mesenchymal Stem Cell Transplantation

Mesenchymal stem cells represent an attractive alternative for fetal therapy. Mesenchymal stem cells from adult bone marrow^{7,8} have been well characterised and are under intensive investigation for therapeutic applications in co-transplantation, muscular dystrophy, myocardial and neural injury, and tissue engineering. They divide rapidly and have high amphotropic receptor levels and are readily transducible with integrating vectors, maintaining transgene expression *in vitro* and *in vivo* without affecting multi-potentiality.^{9,10} Use of mesenchymal

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stem cells should obviate the rejection associated with intrauterine haemopoietic stem cell transplantation. Although immune recognition (MLC, T cell alloreactivity, high NK cells) seems established in humans by the late first trimester, mesenchymal stem cells downregulate certain immune responses,¹¹ which may be advantageous even for autologous use to obviate reactions against transgene products. Adult mesenchymal stem cells suppress T lymphocyte proliferation,^{12,13} and allogeneic/xenogeneic mesenchymal stem cells engraft in a wide range of organs in immunocompetent animal models.^{14,15}

The recent identification of mesenchymal stem cells in the human fetus raises the possibility not only of using a primitive mesenchymal stem cell population, but one which could be harvested for autologous use. Our group has characterised in human fetal blood a population of adherent cells with an immunophenotype comparable to adult mesenchymal stem cells, which self renew over 20 to 40 passages, and differentiate in permissive media along multiple mesenchymal and other lineages including muscle and neural tissue.¹⁶ These circulate only in the first trimester, support haemopoiesis in co-culture, and are similar to comparable populations in fetal liver and bone marrow, suggesting they are stromal cells migrating to sites of definitive haemopoiesis.¹⁶ Consistent with this, they express multiple adhesion molecules,¹⁷ and unlike other fetal cell types are found rarely in maternal blood in pregnancy, leading to the speculation that they engraft maternal organs and may be the cell type responsible for fetomaternal microchimerism.¹⁸ Transplantation of fetal mesenchymal stem cells in a xenogeneic fetal sheep model¹⁴ resulted in low level engraftment in multiple organs, with site specific tissue differentiation.¹⁹ Like adult mesenchymal stem cells, their fetal counterparts are HLA Class 2 negative and suppress mixed lymphocyte reactions.²⁰

Use of fetal mesenchymal stem cells for downstream therapeutic applications obviates some of the ethical and safety concerns (i.e malignant teratoma formation) associated with pluripotent embryonic stem cells on the one hand, while on the other reduces the disadvantage with adult mesenchymal stem cells of limited stem cell potential. Although there remain residual ethical issues with allogeneic use of fetal mesenchymal stem cells from discarded fetal tissue, these should be obviated by collection of autologous fetal blood or liver mesenchymal stem cells in continuing affected pregnancies. Given their ready transducibility (>95% using retroviral and lentiviral vectors),²¹ fetal mesenchymal stem cells may be accessible vehicles for autologous *ex vivo* fetal gene therapy. Fetal interventional techniques under high-resolution ultrasound have already developed to the extent that fetal blood sampling can be performed in ongoing pregnancies as early as 12 weeks with <5% loss rate,²² and advances in imaging and thin gauge fetoscopic cannulation should further render mesenchymal stem cell harvest and reinfusion feasible in the late first/early second trimester.^{23,24} However reports of leukaemia developing following postnatal gene therapy, presumably due to insertional

mutagenesis, currently limit the *ex vivo* gene therapy approach, although this may be able to be overcome by clonal expansion of transduced stem cells after insertional site analysis.

Co-transplantation of adult mesenchymal stem cells has been shown to enhance engraftment of haemopoietic stem cells *in utero* in animal models,²⁵ suggesting that co-transplantation of fetal mesenchymal stem cells may also result in accelerated haemopoietic engraftment following intrauterine transplantation.²⁶

Candidate Diseases

Conditions which may be appropriate for development of intrauterine fetal mesenchymal stem cell therapy include mesenchymal deficiency conditions which improve after postnatal bone marrow transplantation (which contains mesenchymal as well as haemopoietic stem cells) and for which there is a survival advantage for wild type cells. Such approaches first require testing in murine models of genetic disease to demonstrate not only engraftment and tissue-specific differentiation, but also long-term correction of the underlying histological, phenotypic and functional defect.

Duchenne muscular dystrophy is a progressive X-linked myopathy where the absence of dystrophin in muscle fibres leads to damage and regeneration cycles, eventually exhausting the muscle's innate ability to regenerate, resulting in fibrosis. Muscle wasting is apparent by 3 to 5 years with affected individuals wheelchair-bound by 12 years and dying in the third decade from respiratory failure or cardiomyopathy. There is no effective treatment and myoblast transplants have proved disappointing. Skeletal muscle repair after infusion of bone marrow-derived stem cells results in engraftment in up to 10% of muscle fibres,^{27,28} while infusion of adult synovial mesenchymal stem cells in a murine muscular dystrophy model induced synthesis of human dystrophin and decreased abnormal muscle fibres.²⁹ Long-term persistence of donor nuclei in Duchenne muscle has been documented after bone marrow transplantation.³⁰ The utility of a fetal-to-fetal approach is supported by mid-gestation intrauterine transplants in a mouse model of muscular dystrophy with unpurified fetal liver or adult bone marrow, which resulted in widespread albeit low level engraftment and myogenic differentiation.³¹

Another disease for which stem cell-based therapy has been proposed is osteogenesis imperfecta, a disorder of bone fragility and skeletal deformity characterised by defective type I collagen, the major structural protein in bone, which affects 1/10,000 births and for which there is no cure. Already, a few children with osteogenesis imperfecta have been transplanted with allogeneic bone marrow, which engrafted in the genetically defective bone, improving bone mineral content and growth velocity over 3 years follow-up.³² Subsequent therapy using adult mesenchymal stem cells without myeloablation boosted responses further, without apparent immune response in the host.³³ Anecdotally, human fetal liver mesenchymal stem cell transplants have been attempted in a couple of fetuses with

intermediate severity osteogenesis imperfecta, with low level engraftment on preliminary analysis. The development of *ex vivo* gene therapy for osteogenesis imperfecta is more problematic, as it is a dominant negative disorder with therapy needing to suppress the mutant allele in addition to ensuring expression of the wild type allele.

Following animal studies suggesting improvement in the characteristic skeletal and neurological deficit, infusion of bone marrow-derived mesenchymal stem cells in Hurler syndrome is similarly being investigated in affected children and might also eventually be applied *in utero*.³⁴ Niemann Pick disease, a lysosomal storage disorder, is another condition for which mesenchymal stem cell transplants have resulted in histological, biochemical and clinical improvement in murine models.³⁵

Conclusion

Just as gene therapy 10 years ago, stem cell therapy promises widespread application in many branches of medicine including fetal medicine. Theoretical ontological advantages of intrauterine transplantation include increased engraftment and avoidance of immune rejection, as well as treatment before disease morbidity. Human fetal mesenchymal stem cells may have therapeutic application *in utero*, either allogeneic, or as autologous donors of therapeutic genes in inherited deficiency diseases, such as muscular dystrophy, skeletal dysplasias, and inborn errors of metabolism. Understanding stem cell fate after intrauterine transplantation is a necessary prerequisite to further development of this approach in mouse models of debilitating human disease.

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Myogenic Potential of Fetal Mesenchymal Stem Cells

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Introduction

Genetic diseases with intrauterine onset can lead to permanent neurological or musculoskeletal damage in early life. To prevent this, stem cells have been proposed as vehicles for fetal gene or protein delivery because of their ability to expand and give rise to large numbers of multipotent cells. Transplantation in early intrauterine life should capitalise on the ontological opportunity afforded by developing tissues, fetal immune naiveté and the endogenous seeding and engraftment of native stem/progenitor cells, thus raising the possibility that intrauterine therapy may eventually be advantageous for genetic diseases of postnatal onset.¹

Mesenchymal stem cells (MSCs): MSCs represent an attractive alternative for fetal therapy. MSCs from adult bone marrow^{2,3} have been well characterised and are under intensive investigation for therapeutic applications. They divide rapidly and are readily transducible with integrating vectors, maintaining transgene expression in-vitro and in-vivo without affecting multipotentiality.^{4,5}

Human fetal MSC (hfMSC): Human fetal blood contains a population of adherent cells with an immunophenotype comparable to adult MSCs. They proliferate more readily than adult MSCs,⁶ self-renew over 20 to 40 passages, differentiate along multiple mesenchymal lineages (Fig. 1) and have greater potential and differentiate into oligodendrocytes and haemopoietic cells (unpublished data). They circulate only in the first trimester, support haemopoiesis in co-culture and are similar to comparable populations in fetal liver and bone marrow, suggesting they are stromal cells migrating to sites of definitive haemopoiesis.⁷ hfMSCs were transplanted *in utero* at 65 days' gestation in a xenogeneic fetal sheep model to find engraftment in multiple organs, where they underwent site-specific tissue differentiation.⁸

Duchenne muscular dystrophy (DMD): DMD is a progressive X-linked myopathy affecting 1 in 3500 boys.⁹ Muscle wasting is apparent at 3 to 5 years, with affected individuals wheelchair-bound at 12 years and dying in the third decade of life from respiratory failure or cardiomyopathy.¹⁰ In most cases, deletions cause a premature stop codon in the dystrophin gene on chromosome Xp21, which are inherited in two-thirds of cases.¹¹ The genetic defect leads to near absence (<3%) of dystrophin, which otherwise protects muscle from contraction-related damage.¹² There is no effective treatment and myoblast transplants have proven disappointing.¹³

Stem cell therapy for DMD: Several lines of evidence suggest that MSCs participate in regeneration due to muscle damage. Ferrari reported low-level uptake in injured muscle of intravenously administered allogenic bone marrow cells in immunodeficient mice.¹⁴ In mdx¹⁵ mice (a model of dystrophin deficiency) transplanted after lethal irradiation with adult bone marrow cells, Gussoni found dystrophin in up to 10% of muscle fibres. Bittner showed the presence of occasional bone marrow-derived cells in both skeletal

and cardiac muscles in mdx mice.¹⁶ Furthermore, a case report of a boy with DMD and a prior bone marrow transplant for SCID documents long-term persistence of donor nuclei in muscle.¹⁷ Although the responsible cell type is not known, it is likely that these are MSCs, based on Ferrari's finding of greater engraftment of adherent compared to non-adherent marrow cells, and Gussoni's higher levels of dystrophin expression after transplantation with non-purified marrow compared to marrow enriched for HSC. More recently, De Bari transplanted adult human synovial membrane MSCs into both an injury and mdx mouse model to find incorporation into myofibres and restoration of dystrophin expression, respectively.¹⁸ The utility of a fetal to fetal approach is supported by mid-gestation transplants in mdx mice with unpurified fetal liver or adult bone marrow, which resulted in widespread engraftment and myogenic differentiation.¹⁹

We aimed to study myogenic differentiation of hfMSC in-vitro and in-vivo. This would define the conditions whereby myogenesis occurs and may allow the use of lineage committed progenitors in transplantation strategies.

Materials and Methods

hfMSC Samples

After informed consent, first-trimester fetal blood samples were obtained by ultrasound-guided cardiac aspiration between 7 and 13 weeks' gestation at surgical termination of pregnancy. Fetal blood was plated at a density of 10⁵/mL and cultured in 10% fetal bovine serum (Stem Cells, Vancouver). Upon reaching confluence, cells were trypsinised and passaged for cryopreservation or used in the following experiments.

In vitro Myogenic Differentiation

hfMSCs were plated at high densities in fibronectin-coated chamber slides and cultured in a variety of conditions known to promote myogenesis in murine bone marrow stromal stem cells.

1. 5-azacytidine²⁰ (Sigma, UK), a hypomethylation agent, was used as an induction agent at varying concentrations in serum-free conditions.
2. Myoblast-conditioned media²¹ was used at various concentrations in both serum-containing and serum-free media supplemented with additional growth factors (PDGF-BB and EGF, Sigma, UK).

Cells were fixed in methanol/acetone and incubated with monoclonal antibodies to desmin (DAKO, UK) and MF20 (sarcomeric myosin subunit – hybridoma line). Briefly, slides were washed and blocked with 5% albumin and goat serum, and developed using a streptavidin-biotin alkaline phosphatase reaction or a fluorochrome label.

In vivo Work

Immunodeficient adult mouse injections: In order to demonstrate *in*

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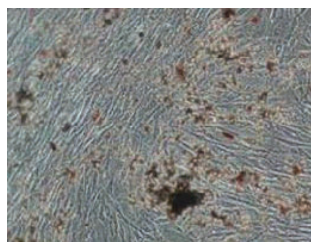


Fig. 1a. Osteogenic differentiation–Von Kossa staining of calcium hydroxyapatite crystals.

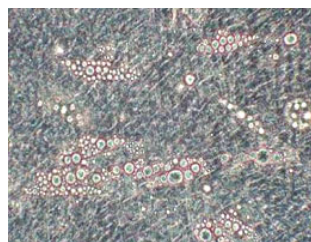


Fig. 1b. Adipogenic differentiation–distinct oil droplets after 21 days in culture.

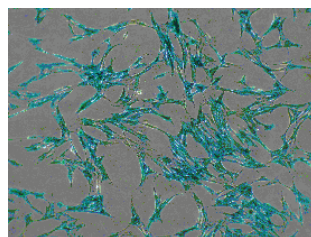


Fig. 1c. X-Gal staining after retroviral transduction with LacZ.

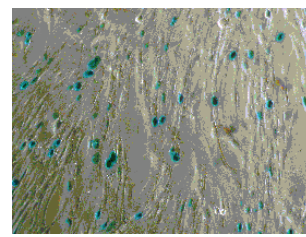


Fig. 1d. X-Gal staining of hMSCs after HIV-1 lentiviral transduction with nuclear localising LacZ.

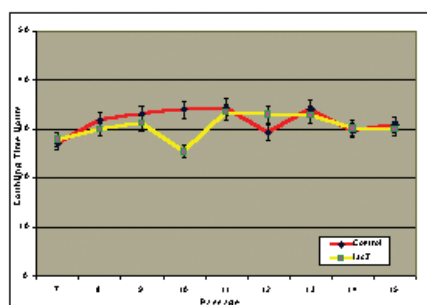
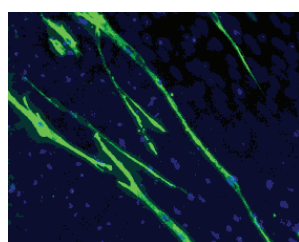
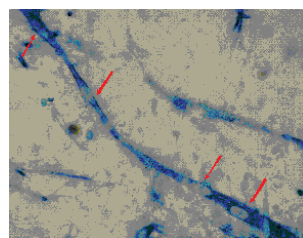


Fig. 1e. Growth kinetics of transduced (yellow) versus mock transduced (red) MSC over successive passages.

Fig. 1. hMSCs – differentiation and viral transduction.



MF20-FITC staining at D12 myogenic differentiation showing multi-nucleated fibres staining positive for MF20 amidst negative cells.



Desmin staining of hMSCs at D14 demonstrating multi-nucleated fibre.

Fig. 2. In vitro myogenic differentiation – MF20 and Desmin Staining.

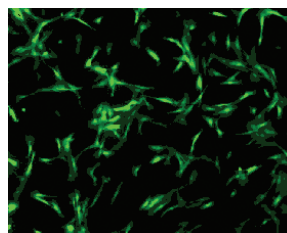


Fig. 1f. GFP expression after lentiviral transduction.

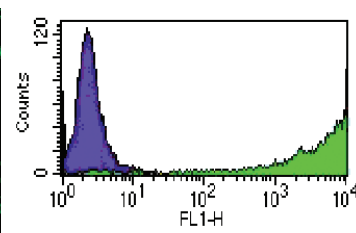


Fig. 1g. FACS data showing transduced (green) versus mock transduced (purple) cells with GFP fluorescence.

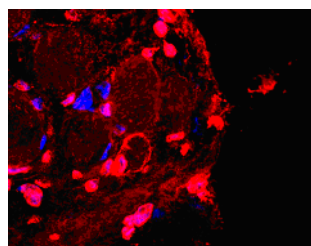


Fig. 3. In vivo adult mice transplants.

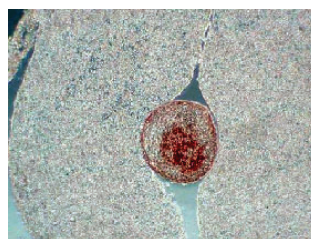


Fig. 4. In utero transplanted.

vivo myogenesis, 5×10^5 low passage hMSCs were injected into irradiated tibialis anterior muscles of immunodeficient c- γ -RAG2 mice.²² Irradiation incapacitates host myogenic cells and possibly releases growth factors,^{23,24} resulting in increased muscle formation from implanted cells.²² After 28 days, the muscle was harvested, snap frozen and sectioned with a cryostat. Sections were blocked with rabbit serum and papain-digested whole fragments of unlabelled secondary anti-mouse immunoglobulins,²⁵ were stained with mouse monoclonal anti-lamin A/C (human nuclear envelope – Novocastra, UK) and human spectrin (human muscle sarcolemmal protein – Novocastra, UK) antibodies, and visualised with a streptavidin-biotin-alexa fluorochrome.

In utero transplantation into MF1 mouse: As a preliminary to *in utero* transplantation work with mdx mice, 5×10^5 hMSCs were transplanted *in utero* into immunocompetent MF1(non-dystrophic) fetal mice. Two days later, they were harvested, formalin fixed, wax-embedded and prepared in 5 μ m sections. Human cells were sought

via immunostaining with a human vimentin (DAKO, UK) and visualised using a peroxidase system (Vector Lab, UK).

Transduction

The retrovirus pBMN-Z-I-Neo expressing LacZ (G. Nolan, Stanford) was transiently transfected into 293T producer cells (ATCC-SD3443) utilising CaPO₄ precipitation.²⁶ Two days after transfection, retroviral supernatant was harvested used for transduction experiments. Early-passages MSCs were plated overnight at low densities 5×10^3 /cm² and subjected to a 3-cycle infection regime. Incubation with retroviral supernatant and polybrene (4 μ g/mL) for a total of 6 hours per cycle was performed. G418 selection and analysis of transgene expression (β -galactosidase) by X-Gal staining was done. Growth kinetics and differentiation potential at short- and long-term time points were performed. Osteogenic and adipogenic differentiation was achieved with standard protocols as published elsewhere.⁷

Because retroviral gene delivery is inefficient, requiring drug

selection,²⁷ multiple rounds of transduction⁴ and prone to expression shutdown,^{27,28} a VSV-G pseudotyped lentiviral vector RRL.ppt.hPGK.GFP.pre.sin-18 (gift from K Brimah and G Cossu) was prepared using transient co-infection with a helper and env plasmid system to 293T producer cells. A single-round infection with polybrene was performed. FACS analysis was done at 48 hours to determine eGFP expression.

Results

In vitro Myogenesis

Unlike murine MSC, chemical induction with a varying concentration of 5-azacytidine for differing periods of time did not promote myogenic differentiation, but led to massive cell death of hfMSCs.

Culturing hfMSCs with myoblast-conditioned media led to the development of desmin-positive cells by day 3 and MF20 positive cells by day 7. By Day 14, large multi-nucleated muscle fibres can be seen that stain positively for both desmin and MF20, indicative of muscle differentiation (Fig. 2).

In vivo Myogenesis

After transplantation into immunodeficient damaged murine muscle, hfMSC participated in regeneration of muscle by forming spectrin positive muscle fibres, albeit at low levels (<1%) – 5 to 6 fibres per muscle section (Fig. 3).

Two days after *in utero* transplantation into immunocompetent MF1 strains, hfMSCs can be detected by staining for human vimentin (Fig. 4).

Transduction

Transduction efficiencies of 44.1% to 48.5% were achieved with the retroviral vector. After G418 selection, the short-term growth kinetics and osteogenic, adipogenic and myogenic differentiation potential remains similar to mock infected control hfMSCs (Fig. 1). On the other hand, lentiviral transduction reached 100% efficiencies as demonstrated by FACS and microscopy.

Discussion

We have shown that hfMSCs can undergo myogenic differentiation under permissive conditions in-vitro, and in an immunodeficient mouse model form muscle, in response to environmental cues, albeit at low levels. However, this mouse model has only low levels of muscle regeneration compared to the dystrophic mouse (mdx) which approximates human DMD. Hence, there is little selective pressure for the transplanted cells.

Identification of human cells two days after injection into E14 immunocompetent MF1 mice provides proof-in-principle that they survive in the xenogenic fetal environment. Analysis of post injected mice at longer time intervals in the near future will allow us to determine the site(s) of engraftment, particularly whether they contribute to muscle regeneration in dystrophic mdx mice. hfMSCs are readily transducible with high efficiencies using a lentiviral construct.

This work evaluates a novel type of MSCs for allogeneic or even autologous fetal therapy of not only debilitating childhood muscle diseases, such as muscular dystrophy, but a range of other mesenchymal deficiency conditions as well. With advances in early fetal blood sampling techniques, *in utero* treatment of genetic diseases can be treated with genetic manipulation of autologous hfMSCs.

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Subjective versus Observer-rated Insight in First-Episode Psychosis in Singapore

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Introduction

Many patients with psychotic disorders lack insight into their illness and this has been suggested to be a manifestation of the illness itself, rather than a coping strategy.¹ Most of the studies on insight have focused on the clinical correlates of poor insight, such as psychopathology,²⁻⁴ neuropsychological impairment^{2,5} and structural brain damage.⁶ Studies have reported an association between lack of insight and a poor outcome of the illness,^{7,8} as well as with non-compliance with treatment.⁹⁻¹¹

Insight is viewed as a complex and multi-dimensional phenomenon that includes elements of a psychological, psychopathological, neurocognitive and interactional nature.³ However, results from previous studies on insight have been largely inconclusive. This was documented in a meta-analysis⁴ of 40 published English language studies to determine the magnitude and direction of the relationship, or effect size, between insight and symptom domains in schizophrenia, and to determine moderator variables that were associated with the variations in effect sizes across studies. Limitations have been attributed to difficulties with defining and measuring levels of insight in this population.¹² Insight is usually assessed with rating instruments, which may be either a clinician or an observer-rated insight scale,¹³ or a self-rated scale.¹⁴ The latter is not so extensively used, possibly because these self-rated instruments are assumed to be incapable of adequately capturing how patients understand and feel about their illness.¹⁵ However, studies have shown that patients with psychosis are able to use a range of different frameworks to understand and explain their experiences.¹⁶

To date, there has been no study to compare and elicit the differences (if any) between subjectively reported insight and objectively reported insight. The present study aimed to study this relationship in Asian patients with first-episode psychosis.

Materials and Methods

Sample

The study population comprised 98 consecutive patients between the ages of 15 and 40 with first-episode psychosis and who are able to read and write English. Individuals with current substance abuse, mental retardation, neurological disorder or any other medical illness were excluded.

Approval for the present study was obtained from the Research and Ethics Committee of the Institute of Mental Health. All patients provided written informed consent.

Procedure and Measures

The diagnosis was established using the Structured Clinical Interview for DSM-III-R (Patient Version) (SCID-P).¹⁷ The Duration of Untreated Psychosis (DUP) was defined as the time interval between the onset of psychotic symptoms (hallucinations, delusions, thought disorder and/or disorganised behaviour) and hospitalisation for psychosis or initiation of adequate treatment.¹⁸

The severity of psychopathology was assessed with Positive and

Negative Syndrome Scale for Schizophrenia (PANSS),¹⁹ and the quality of life was assessed with the World Health Organization Quality of Life-Bref (WHO-QoL BREF).²⁰ This is a 26-item self-administered subjective measure of quality of life. Its multidimensional nature allows for 4 domains of assessment: physical health, psychological functioning, social relationships and environment. High scores indicate good QoL.

Insight was assessed with the Birchwood Insight Scale¹⁴ which is an 8-item self-report measure of subjective insight based on the awareness of illness, need for treatment and attribution of symptoms. Scores range from 0 to 12, with low scores indicating reduced insight. All the patients were also assessed by a research psychologist with the Scales to Assess Unawareness of Mental Disorder (SUMD).^{7,13} This scale assesses the awareness of the illness, response to medication, social consequences of having a mental disorder, and specific symptoms of the illness. High scores on this scale indicate less insight. Interrater reliability for the PANSS and the SUMD was >0.8.

Statistical analyses were carried out using SPSS 11.0. Normality assumption of quantitative data was checked using Kolmogorov

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF STUDY PATIENTS (n = 98)

Variable	No. of patients (%)	
Gender		
Male	57	(58.2)
Female	41	(41.8)
Race		
Chinese	68	(69.4)
Malay	23	(23.5)
Indian	6	(6.1)
Others	1	(1.0)
Religion		
Buddhism/Taoism	47	(47.9)
Christianity	13	(13.3)
Islam	24	(24.5)
Hinduism	2	(2.0)
No religion	12	(12.2)
Marital status		
Single	66	(67.3)
Married	24	(24.5)
Divorced/Separated	7	(7.2)
Widowed	1	(1.0)
Education		
Primary	14	(14.4)
Secondary	38	(38.7)
Tertiary	30	(30.6)
Vocational institute	10	(10.2)
Pre-university	6	(6.1)
Living situation		
Alone	7	(7.1)
With husband/wife	25	(25.6)
With parents/siblings	66	(67.3)
Employment		
Employed	26	(26.6)
Unemployed	42	(42.9)
Student	12	(12.2)
Homemaker	12	(12.2)
National service	6	(6.1)

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TABLE II: CORRELATIONS BETWEEN SUBJECTIVE INSIGHT (BIRCHWOOD INSIGHT SCALE) AND QUALITY OF LIFE (WHO-QoL)

	WHO-QoL domains				
	Physical Health	Psychological Health	Social Relationship	Environmental Health	Total QoL score
Birchwood Insight domains					
Relabel symptoms	-.278**	-.283**	-.300**	-.255*	-.314**
Awareness of illness	-.229*	-.208*	-.183	-.186	-.248*
Need for treatment	-.227*	-.061	-.042	-.112	-.148
Total Birchwood score	-.330**	-.257*	-.247*	-.253*	-.325**

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Smirnov 1 sample test. Pearson's correlation coefficients were calculated to study the relationship between objective and subjective measures of insight if normality assumptions were satisfied otherwise Spearman's correlation will be presented. A multiple regression was performed on the total WHO-QoL score using age, gender, ethnic group (Chinese vs non-chinese), education (tertiary vs non-tertiary), employed vs unemployed, living alone or not, DUP since onset of symptoms, total Birchwood score, total PANSS score and total SUMD score.

Results

The mean (SD) age of the 98 patients was 28.9 (7.1) years. The mean (SD) DUP was 21.5 (35.7) months; the median DUP was 3 months. Forty-two (42.9%) of the 98 patients met DSM-IV criteria for schizophrenia, 24 (24.5%) for schizophreniform disorder, 4 (4.1%) for schizoaffective disorder, 12 (12.2%) for brief psychotic disorder, 7 (7.1%) for psychotic disorder not otherwise specified and 9 (9.2%) for mood disorders with psychotic features. Demographic characteristics are described in Table I.

There was no significant relation between objectively-rated illness awareness with subjective measures of insight ($r = -0.094$, $p = 0.361$; Spearman's correlation) (Fig. 1).

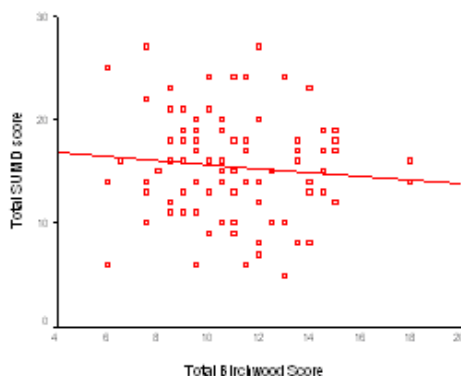


Fig. 1. Scatter plot of total SUMD score against total Birchwood score.

None of the objective and subjective insight items were found to be associated with psychopathology. Significant relationships were found between domain scores of the Birchwood Insight Scale and the WHO-QoL (correlation coefficients are presented in Table II).

The following predictors, age, gender, ethnic group (Chinese vs non-chinese), education (tertiary vs non-tertiary), employed vs unemployed, living alone or not, DUP since onset of symptoms, total Birchwood score, total PANSS score and total SUMD score, were modeled in a multiple regression analysis on total WHO-QoL. Only the total Birchwood score was significant with every unit increase in Birchwood score, the WHO-QoL decreases by 2 units (95% CI 0.6 to 3.6, $p = 0.007$).

Discussion

Our findings suggest that there is no agreement between subjectively rated insight and the observer-rated insight. As insight has been conceptualised as a multi-dimensional construct,³ the two instruments may actually be measuring different dimensions of insight. As the symptomatology of schizophrenia is also multi-dimensional, it is not surprising that we did not find a significant association between insight and psychopathology, as the instruments used in our study may be relatively simple and may not capture the complexity of insight and psychopathology of schizophrenia. This is consistent with the findings of previous studies,^{7,10,11} which reported no significant association between insight, acute psychopathology and negative symptoms. This has led to the suggestion that further studies should examine how specific dimensions of insight relate to other symptoms in psychotic illnesses.⁴

A limitation of our study is that we did not assess the level of neurocognitive functioning in this group of patients. The neuropsychological view of insight is that awareness of illness may be a higher-order cognitive ability,⁶ and previous studies have hypothesised that poor insight in psychosis could largely be a function of impaired information processing capacities.^{2,6}

Although we have included only English-speaking patients, there is also a need to validate these instruments in our local population as cultural factors would determine the perception of the presence of psychiatric symptoms, and the reporting of these symptoms may also vary from culture to culture.²²

Our findings suggest that objective insight ratings alone may be insufficient to adequately account for the level of awareness that a patient has about the psychotic illness. As such, future studies should incorporate different scales measuring different dimensions in the assessment of insight, and take into consideration the cultural nuances of the study population.

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Young Investigator's Award Finalist

Usefulness of Serum Soluble CD38 and CD157 Levels in Differentiating SLE, RA and Healthy Adults and their Relationship with Disease Activity

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Introduction

Human CD38 is a transmembrane glycoprotein involved in lymphocyte activation and adhesion to endothelium. The ecto-cellular domain of the molecule possesses properties of a bi-functional enzyme catalysing both the synthesis from NAD⁺ and the hydrolysis of the calcium-releasing metabolite cyclic ADP-ribose (cADPR).¹⁻³ In addition to the existence of a transmembrane CD38, a soluble form of CD38 (sCD38) is detectable in the cell culture supernatant of allo-activated T lymphocytes and of several tumor cell lines and also *in vivo*. It is assayable in normal (fetal serum and amniotic fluid) and pathological (serum and ascites from patients with multiple myeloma, and serum from patients with AIDS) biological fluids.⁴

Human CD157 is a glycosylphosphatidylinositol-anchored membrane protein, with ADP-ribosyl cyclase/cADPR hydrolase activity, having 30% homology with CD38. The expression patterns of both molecules are overlapping in several tissues and haemopoietic systems, suggesting that CD157 and CD38 are functionally co-operating or compensating in certain situations.⁵ Bone marrow nurse-like cells in patients with rheumatoid arthritis (RA), which express greater amount of CD157, were demonstrated to be able to rescue B-cells from spontaneous apoptosis.⁶ Similarly, soluble CD157 (sCD157) has also been noted in the serum of patients with RA.⁷ It was proposed that very high level of sCD157 might signify severe RA disease in RA.

Objectives

In inflammatory conditions or autoimmune diseases, soluble forms of membrane proteins have been detected in sera and clinical significance has been reported.^{8,9} We aimed to measure serum sCD38 and sCD157 in patients with RA and systemic lupus erythematosus (SLE), and to identify their association with disease activity.

Materials and Methods

Serum samples were obtained from 120 patients with SLE and 90 patients with RA, who were consecutively seen in the outpatient rheumatology clinics between December 2000 and May 2001, after informed consent. All RA and SLE patients fulfilled the American College of Rheumatology criteria for RA¹⁰ and SLE¹¹ respectively. Control serum samples were obtained from 56 consented healthy donors. Disease activity was assessed using Disease Activity Score using 28-joint count (DAS 28) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) for RA and SLE, respectively. Disability in RA was assessed using modified Health Assessment Questionnaire (mHAQ).

sCD38 and sCD157 levels were measured using enzyme-linked immunosorbent assay (ELISA), which utilised specific monoclonal antibodies for the identification of molecules of interest. The levels were expressed in optical density units (ODU).

Continuous data are presented in the form of mean $\pm$ standard

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deviation or median and range. Categorical data is presented as percentage. Analysis of the data was done using the Inter-cooled STATA 7.0 for Window. Kruskal-Wallis test, Mann Whitney U test, and Spearman's rank correlation were performed as indicated. Significance level was set at 0.05. Receiver operating characteristic (ROC) analysis was used to assess the discriminating power of sCD38 and sCD157 at various levels.

Results

A total of 90 RA and 120 SLE patients were recruited. Mean age of RA and SLE patients was 49.1 ± 10.7 years and 37.2 ± 11.3 years, respectively. Female constituted 85.0% and 93.4% of RA and SLE patients, respectively. Chinese formed 77.4% of RA patients and 76.9% of SLE patients. The mean disease duration was 4.7 ± 4.6 years in RA (median 3.5 years) and 5.9 ± 5.0 years in SLE (median 5 years). The DAS 28 score of RA patients was 3.7 ± 1.3 . SLE patients had a median SELDAI of 4 (range 0 to 24).

sCD38 levels were significantly different among SLE, RA and healthy donors, with the levels being lowest in SLE and highest in healthy donors (0.074 ± 0.043 ODU for SLE versus 0.122 ± 0.044 ODU for RA versus 0.157 ± 0.005 ODU, $p < 0.0001$).

sCD157 was undetectable in 4% of healthy controls, 21% of RA and 5% of SLE ($p < 0.0001$). Furthermore, sCD157 levels in RA identified a subgroup of patients with very high levels of sCD157 (35,000 ODU in 23% of cases; mean levels: $35,280 \pm 220$ ODU) compared to the rest of the group ($14,376 \pm 1317$ ODU). None in normal healthy controls and only 2 patients with SLE had such high levels. However, the disease activity as assessed using DAS 28 among those with high levels did not differ significantly from the rest (3.7 ± 1.7 and 3.6 ± 1.4 , respectively). Similarly, median mHAQ score did not show significant differences between the 2 groups (0.125 versus 0.5).

Discussion

Our study showed significantly suppressed sCD38 in both SLE and RA relative to the levels in healthy adults. This was initially surprising since activated T and B cells in these 2 autoimmune diseases are well known and membrane surface CD38 expression is up-regulated in activated lymphocytes.¹² The results of our other study (unpublished data), which showed increased production of autoantibody to CD38 offered a possible explanation to the suppressed sCD38 levels found. In parallel with endocytosis, shedding of CD38 is a regulatory element in activation and adhesion processes mediated by CD38. Internalisation of CD38 is triggered off by both agonistic and non-agonistic monoclonal antibodies.¹³ With the elevated anti-CD38 antibody production and, thus, internalisation process, reduced shedding of sCD38 is expected in SLE and RA. This further supported by the fact that in SLE, where the production of anti-CD38 autoantibody is highest (unpublished data), sCD38 level is the lowest. The difference in sCD38 levels in various conditions allows us to differentiate healthy adult, RA and SLE patients, as shown with ROC analysis. This differentiation is especially useful in situation where SLE presented as rheumatoid-like arthritis or in RA with positive ANA.

We also confirmed the previous finding of the elevated sCD157 levels in RA with a subgroup of patients with extremely high levels

and its lack of relationship with the disease activity as assessed using DAS 28.⁷ However, different from the previous report, we did not find very high level of sCD157 among those with severe disease as determined by disability. Furthermore, in our study, SLE had comparably high level as in RA, even after those with very high levels (≥ 35000 ODU) were excluded. It is recognised now that B-cells also play an important pathogenetic role in RA, as in SLE. Our finding is, thus, not surprising and might suggest that sCD157, as a molecule which salvages B-cells from apoptosis,⁵ plays an important role in the pathogenesis of both diseases.

Conclusion

Suppressed serum sCD38 levels and raised serum sCD157 levels were found in RA and SLE. The levels of sCD38 may be helpful in differentiating SLE, RA and the healthy state. sCD38 levels correlate significantly with disease activity in RA, but not in SLE. High level of sCD157 is more likely to be found in RA. Our study did not find any relationship between disease activity in RA and sCD157 level.

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Real-time Quantitative Polymerase Chain Reaction (RQ-PCR) using the LightCycler: A Rapid, High-throughput Method for Detecting and Quantifying Fusion Transcripts in Childhood Leukaemias for Disease Stratification and Prognostication

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Introduction

Cancer is a genetic disease. Its uncontrolled growth is driven by underlying genetic mutations that prevent differentiation and cause relentless cell divisions and apoptotic death. The genetic basis for leukaemogenesis is well elucidated and specific mutations can be found in up to 50% of patients.

As drivers of leukaemogenesis, oncogene fusions are the most accurate diagnostic markers and predictors of treatment outcome. It is, hence, crucial in modern leukaemia therapy to detect these fusion transcripts so that the correct treatment intensity can be applied to improve outcome and decrease side effects. Conventional cytogenetics, the gold standard, is laborious, laboratory-dependent and completely misses submicroscopic translocations like t(12;21)/TEL-AML1.¹

Reverse-transcription polymerase chain reaction (RT-PCR) can detect these oncogene fusions but is also time-consuming and requires post-PCR handling, greatly increasing the risk of PCR-product contamination.² Moreover, it is a qualitative assay and cannot quantify transcripts for monitoring disease post-therapy.

Early response to therapy as determined by Ig/TCR rearrangements, is one of the most important prognostic markers of ALL therapy.³⁻⁶ Unfortunately, they require high levels of diagnostic blasts to identify a leukaemia-associated marker and are susceptible to clonal evolution. We postulate that fusion transcripts are not constrained by these parameters and can complement Ig/TCR for disease stratification.⁷

Real-time quantitative PCR (RQ-PCR) is a new molecular platform that allows specific quantitation of gene copy numbers. It is rapid, has high-throughput and does not require post-PCR handling.^{7,8}

Here, we studied the feasibility of using RQ-PCR to detect and quantify fusion transcripts in childhood leukaemia for diagnosis and prognostication.

Materials and Methods

RNA extraction and Reverse Transcription

Bone marrow (BM) aspirates were collected in EDTA, isolated by density gradient centrifugation and total RNA extracted using TRIzol reagent. cDNA was synthesized with random hexamers using the Omniscript Reverse Transcriptase kit. Primers and probes were obtained from published data.^{9,10}

Qualitative Detection of Fusion Transcripts

Multiplex RT-PCR setup. This was designed to screen patients for the commonly reported splice variants of TEL-AML1, E2A-PBX1,

MLL-AF4 and BCR-ABL p210 transcripts. RQ-PCR was performed on a LightCycler Instrument using Taqman probes.

Positive, negative and no-template controls were included in each run by replacing the template DNA with positive cell lines for the specific fusion transcripts, total RNA from HL60 cell line or PCR-grade water, respectively. Positive multiplex panels underwent split-out PCR with individual primers to confirm the specific splice variant.

Quantitative Monitoring of Fusion Transcripts

Standard curves were prepared using 10-fold dilutions of plasmids containing the specific fusion transcripts cloned into pCR® II-TOPO vector. GUS was selected as the housekeeping gene to compensate for variations in sample volume, target recovery during RNA extraction and inhibitors.

Results

Qualitative Screening

A total of 107 diagnostic or relapse BMs were screened for the common fusion transcripts in childhood leukaemia. Twenty-eight (26%) patients tested positive for fusion transcripts (Table I). RQ-PCR results correlated perfectly with conventional RT-PCR and even detected a case originally missed by the latter.

Quantitative Studies

Quantitative assays were performed on 41 BM samples from 12 patients (Table II), including 10 diagnostic, 2 relapse and 29 follow-

TABLE I: RESULTS OF QUALITATIVE SCREEN FOR ONCOGENE FUSIONS

Diagnosis	Fusion transcript	Positive cases n = 28	Percentage affected	Published data ¹ (%)
ALL n = 86	TEL-AML1	12	14	25
	MLL-AF4	3	4	2
	BCR-ABLp190	5	6	5
	BCR-ABLp210	*	1	0.5
	E2A-PBX1	0	0	5
	Total	21	24	25-50
AML n = 18	CBFβ-MYH11	1	6	10
	AML1-ETO	3	17	14
	Total	4	22	20-40
CML n = 3	BCR-ABLp210	3	100	>95
Overall n = 107		28	26	

*biphenotypic leukaemia

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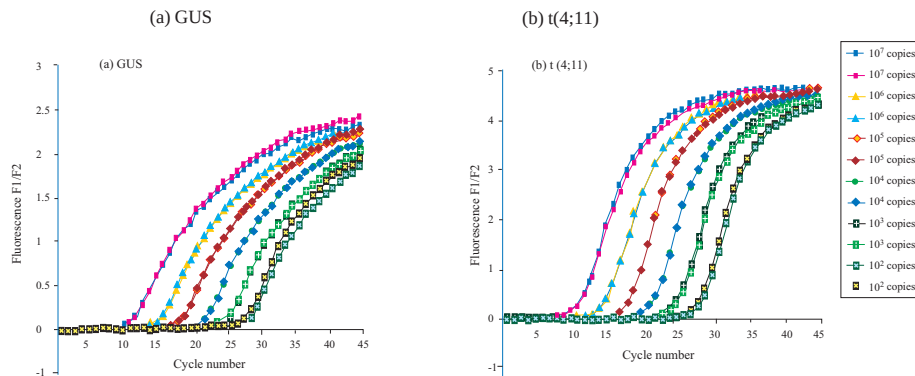


Fig. 1. Representative graphs showing increase in fluorescence during amplification of sequences from 10^2 - 10^7 copy numbers of (a) GUS and (b) t(4;11) plasmid DNA.

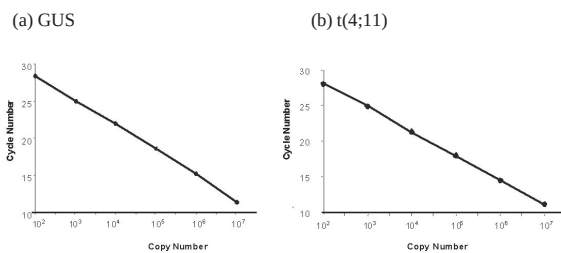


Fig. 2. Representative graph showing linear relationships between crossing point and starting copy number of cDNA standards. 10^2 - 10^7 copies of GUS plasmid cDNA were amplified for 40 cycles. The slopes were -3.424 cycles/log decade. The slope of the standard curve was converted to amplification efficiency E by the following algorithm: $E = 10^{-1/\text{slope}}$ (E should not differ by more than 1.5-2.2). The regression coefficients were -1.00 for all runs.

up samples. The median duration of follow-up was 65 days (range 1-12 months).

Standard Curves⁷

Regression coefficients were -1.00 (ideal -1.00) for all runs. The mean $\pm$ SD slope was -3.41 ± 0.15 (ideal -3.3) with mean $\pm$ SD intercept of 36.01 ± 2.15 . For the GUS standard curves, slope was -3.44 ± 0.02 and intercept 35.64 ± 0.27 (Figs. 1 & 2).

Sensitivity

The detection limit of each assay was 10-50 fusion transcript copies.

Reliability of Assay

Intra-assay variability analysis was determined using ten-fold

serial dilutions of a positive control sample (cell line cDNA) in triplicates from 100% to 0.01% of template concentration. Inter-assay variability was studied by repeating the analysis of the same samples in 3 consecutive assays. The inter- and intra-assay comparisons, expressed as crossing thresholds (CTs), showed good reproducibility with less than 0.93 cycle difference, reflecting a coefficient of variation of less than 3%.

Fusion Transcript Quantitation in ALL (Table III)

Four (67%) of six *TEL-AML1* patients showed complete transcript clearance by day 33 while 2 (33%) had low-level disease (normalised ratio 8×10^{-5}).¹¹⁻¹³ These correlated perfectly with the Ig/TCR method of minimal residual disease (MRD) detection:¹⁴ the 2 patients with low-level disease had MRD of 2×10^{-4} and 7×10^{-4} , respectively.

Similarly, both patients with *MLL-AF4* transcripts demonstrated clearance by day 33, although 1 had intermediate-level disease (3×10^{-3}) by Ig/TCR MRD levels.

For the patient with *BCR-ABLp190*-positive ALL, this transcript persisted at day 33 and even 6 months later. There was, however, a 2-log reduction of MRD from 63,510 to 636.8 copies/uL cDNA (normalised ratio 6.57 to 0.04) by day 33. Using Ig/TCR markers, she had intermediate MRD at day 33 and clearance of disease at 5 months. This suggests that fusion transcripts are potentially more sensitive than Ig/TCR rearrangements for MRD detection.

Fusion Transcript Quantitation in AML

Neither AML patient showed transcript clearance after the first course of chemotherapy. The *CBF β -MYH11* transcripts demonstrated a 4-log reduction between diagnosis and treatment cessation¹⁵. The *AML1-ETO* transcripts showed a 3-log reduction after 2 courses of chemotherapy.¹⁶

TABLE II: QUANTITATION STUDIES COMPARING ONCOGENE FUSION TRANSCRIPTS AND IG/TCR MARKERS

Patient	Diagnosis (FT)	Fusion transcript at diagnosis	FT/Gus after induction tx	FT/ Gus after induction tx	MRD level (Ig/TCR)	Clinical progress	No.
1	B-ALL	<i>TEL-AML1</i>	1.53	0.00000	Not done	CR* at 7.5 months	
2	B-ALL	<i>TEL-AML1</i>	2.12	0.00000	$<5 \times 10^{-5}$	CR at 5.83 month	
3	B-ALL	<i>TEL-AML1</i>	1.26	0.00000	$<1 \times 10^{-4}$	CR at 3.77 months	
4	B-ALL	<i>TEL-AML1</i>	2.78	0.00008	2×10^{-4}	CR at 3.27 month	
5	B-ALL	<i>TEL-AML1</i>	6.91	0.00008	7×10^{-4}	CR at 7 months	
6	B-ALL	<i>TEL-AML1</i>	1.55	0.00000	$<2 \times 10^{-5}$	CR at 2.1 months	
7	B-ALL	<i>MLL-AF4</i>	4.12	0.00000	3×10^{-3}	CR at 6.43 months	
8	B-ALL	<i>MLL-AF4</i>	1.16	0.00000	$<5 \times 10^{-5}$	CR at 4.07 months	
9	B-ALL	<i>BCR/ABLp190</i>	6.57	0.25763	8×10^{-4}	CR at 8.03 months	
10	AML-M4	<i>CBFβ-MYH11</i>	2.13	0.00177	Not done	CR at 8.47 months	
11	AML-M2	<i>AML1-ETO</i>	3.34	0.00076	Not done	CR at 3.73 months	
12	CML	<i>BCR/ABLp210</i>	NA**	NA**	NA**	Deceased	

* CR clinical remission

** The CML patient's BM samples were timed differently. Please refer to Figure 3.

TABLE III: SUMMARY OF SENSITIVITY OF VARIOUS FUSION TRANSCRIPTS USED FOR MRD QUANTITATION

Fusion transcript	No. of patients	At diagnosis/relapse		At Day 33 (end of induction remission treatment)*	
		Transcript Copies/ uL cDNA Median (range)	Normalised with GUS Median (range)	Transcript copies/ uL cDNA Median (range)	Normalised with GUS Median (range)
<i>TEL-AML1</i>	6	91225 (38720-217100)	1.84 (1.26 - 6.91)	0.60 (0.00 - 3.96)	0.00004 (0.00000-0.00012)
<i>MLL-AF4</i>	2	42255 (40690-43820)	2.64 (1.12- 4.12)	0.00	0.00000
<i>BCR-ABLp190</i>	1	63510	6.57	4153.00	0.25763
<i>CBFβ-MYH11</i>	1	34710	2.13	67.43	0.00177
<i>AML1-ETO</i>	1	366300	3.34	105.30	0.00076

* In AML patients, a repeat BM was performed before commencing on the second course of chemotherapy rather than on day 33.

† Data for the CML patient is presented separately.

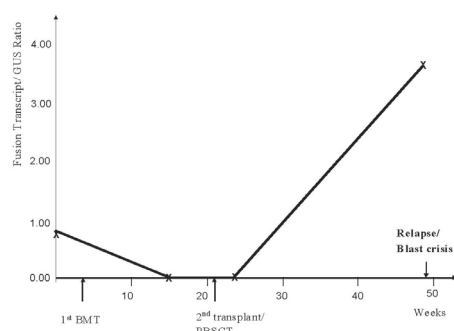


Fig. 3.

BCR-ABL Levels and Clinical Progression¹⁷

Transcript levels of the CML patient correlated well with his clinical course. There was complete clearance after his first BMT, low transcripts (7×10^{-3}) after his second transplant which increased to 3.57 at relapse (Fig. 3).

Correlation with Cytogenetics

Our results correlated with conventional karyotyping where available. *TEL-AML1* was missed entirely by the latter.

Discussion

As the local cure rates for childhood ALL and AML improve to 75% and 50%, respectively, it is clear that leukaemia is a highly heterogeneous disease. Fusion transcripts are extremely useful for disease stratification as they accurately identify different risk groups. For example, *TEL-AML1* patients have 90% EFS, while *BCR-ABL* and *MLL-AF4* patients have less than 20% EFS. Similarly, for AML, *AML1-ETO* and *CBFβ-MYH11* have excellent outcome (70% EFS) on our therapy. It is, hence, crucial to incorporate the detection of oncogene fusion screening for modern management of leukaemia.

We have demonstrated that RQ-PCR is superior to conventional karyotyping and RT-PCR as a screening tool for fusion transcripts in childhood leukaemia.¹⁸⁻²¹ Conventional karyotyping, only successful in 60% to 80% of cases, is highly laboratory-dependent and completely misses submicroscopic fusions like the common *TEL-AML1* fusion gene (14% of ALL cases here).

RQ-PCR has a much faster turnaround time (7h versus 13h versus 1 week) and a higher maximal throughput (29 versus 6 versus 4 screens per technician day) compared to RT-PCR and cytogenetics, respectively. This is crucial for large studies like our Malaysia-Singapore ALL 2003 study, where timely delivery of results is vital.

RQ-PCR permits accurate quantitation of PCR products during the exponential phase of the amplification process, in contrast to end-point quantitation in classical PCR, making it a highly valuable

quantitative tool.⁷ The intra- and inter-assay reproducibility is excellent in our study.

Conventional microscopy is very insensitive: $<5 \times 10^{-2}$ (5 blasts in 100 nucleated cells) is considered complete remission. We have previously demonstrated that IgH MRD markers increase sensitivity of detection to 5×10^{-5} , a 3-log improvement compared to conventional microscopy. Moreover, IgH-based MRD levels at 1 month of therapy was highly correlated with outcome.²²

To determine if fusion transcripts have comparable MRD quantitative capabilities as IgH/TCR markers, we compared MRD determination using both techniques for 8 patients with oncogene fusion transcripts. Both methods have comparable sensitivities with complete concordance in 6 cases. In one case of *MLL-AF4*, fusion transcripts were undetectable despite intermediate-level disease by IgH, while the reverse was true for a case of *BCR-ABL*-positive ALL, where fusion transcripts showed persistent disease²³ whilst IgH was negative.

In this study, 24% of ALL cases carried fusion transcripts while 97% carried IgH/TCR gene rearrangements. In AML and CML, it was informative in 22% and 100% of cases, respectively, compared to 0% in IgH/TCR. Fusion transcripts, thus, play a bigger role in the monitoring of these conditions as Ig/TCR is not useful. *BCR-ABLp210* has been used to monitor response to BMT. Patients who are persistently PCR-negative have a low risk of relapse. Conversely, return of PCR positivity often heralds relapse.¹⁷

IgH/TCR methodology requires high blast counts at diagnosis to identify a marker, making it unsuitable for previously treated patients. Fusion transcripts are present in large numbers at diagnosis and have high sensitivities of 10-50 copies. They should, thus, be detectable 1-2 weeks after commencing therapy. It is a robust marker as it does not undergo evolution with treatment, in contrast to Ig/TCR gene rearrangements. Fusion transcript quantitation is, thus, highly complementary to IgH/TCR MRD risk stratification. This will be explored in our current Malaysia-Singapore ALL 2003 trial.

Conclusion

We conclude that RQ-PCR detection and quantitation of fusion transcripts is a new and important tool in the armamentarium of the paediatric oncologist. It supersedes conventional RT-PCR and karyotyping in qualitative assays and complements current methods of MRD detection using Ig/TCR gene rearrangements. In AML and CML, fusion transcript quantitation is the MRD marker of choice. In addition, it is a highly efficient tool with a fast turnaround time and increased throughput.

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Young Investigator's Award Finalist

Angiomyogenesis using Human Myoblast Carrying Human VEGF₁₆₅ for Injured Heart

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Introduction

Therapeutic angiogenesis using vascular endothelial growth factor (VEGF) for the treatment of ischaemic heart disease has been studied in animal models and clinical trials.^{1,2} The common modes of VEGF delivery include direct introduction of angiogenic protein and injection of plasmid or recombinant viral vectors.³ Recent studies have suggested therapeutic angiogenesis through cell-mediated gene transfer.⁴ The transplantation of genetically modified cells serves as a reservoir to produce biologically active angiogenic factors in a localised and sustained pattern.

Skeletal myoblast, the precursors of skeletal muscle fibres, can be induced to proliferate, propagate and differentiate *in vitro*. Recently, the promising results for improved heart function using myoblast transplantation have generated considerable interest amongst researchers.⁵ Myoblast transplantation improves both *in vivo* and *ex vivo* indices of global ventricular dysfunction and prevents deleterious remodelling to achieve global myocardial function after myocardial infarction. Considering skeletal myoblast is a good carrier for gene therapy, the genetically engineered skeletal myoblast has been studied for cardiac repair.⁴

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In the present study, we assessed the efficacy of genetically engineered human skeletal myoblast carrying human VEGF₁₆₅ (HSM-VEGF₁₆₅) in a rodent heart model of cryo-injury. We hypothesised that this combined strategy could achieve concurrent angiogenesis and myogenesis.

Materials and Methods

Cell Culture

The purity of myoblast was assessed by desmin immunostaining. Human umbilical vein endothelial cell line (HUVEC), HEK-293 cells and FLYA-4 cell lines were cultured in respective basal medium supplemented 10% FBS.

Propagation of Adenovirus Vector Carrying hVEGF₁₆₅ and Transduction of Myoblast

Adenovirus vector carrying hVEGF₁₆₅ (Ad-hVEGF₁₆₅) was propagated using HEK-293 cells. At 70% confluence, the cells were infected with adenoviral vector carrying VEGF₁₆₅ (Ad-VEGF₁₆₅). At the stipulated time, the supernatant from HEK-293 cells was removed, centrifuged and used for transduction of myoblast.

Myoblasts were propagated in laminin coated tissue culture flasks. The cells were exposed to virus medium for 8 hours followed by refresh Super medium. The transduction procedure was repeated thrice with 24 hours' interval each time.

Characterisation of Ad-VEGF₁₆₅ Transduced Myoblast In vitro Immunohistochemical Staining of Myoblast

Myoblasts transduced with Ad-hVEGF₁₆₅ (HSM-VEGF₁₆₅) were grown on glass slides and fixed with -20°C cold methanol. After blocking with 1% BSA and incubating with 3% H₂O₂, cells were washed with PBS and incubated with 1:500 dilution of anti-VEGF₁₆₅ overnight followed by 1: 200 goat anti-rabbit IgG conjugated with HRP. The VEGF-positive cells were observed and visualised with DAB visualisation system.

Enzyme-linked Immunosorbent Assay for hVEGF₁₆₅

The detection of hVEGF₁₆₅ secreted from myoblast transduced with Ad-VEGF₁₆₅ (M-VEGF₁₆₅) was carried out using hVEGF₁₆₅ Sandwich ELISA kit (Chemicon Inter., USA) according to the manufacturer's instructions.

RT-PCR Analysis for hVEGF₁₆₅ Gene Expression in Transduced Human Skeletal Myoblast

The total RNA was isolated by using Total RNA Isolation Kit (PURESCRIPT USA). The RT-PCR analysis of isolated mRNA was done using QIAGEN One-step RT-PCR Kit (QIAGEN). The RT-PCR primers of hVEGF₁₆₅ 5'→3' (5' ATGAACCTTCTG CTGTCTTGGGTG 3') and 3'→5' (5' TCACCGCTCGGCTTG TCACA 3') and human GAPDH were 5'→3' (5' GTGGAGGAGT GGG TGTCGCTG 3') and 3'→5' (5' CGGATTTGGTCGTATT GGGCG 3') were used for amplification.

Thymidine [³H] Incorporation Assay

HUVEC (1x10⁵ cell / well) were seeded in 6-well plates in triplicate for each sample. After 12 hours' culture with DMEM containing 2% FBS, the cells were cultured with conditioned medium from various samples. Each well was supplemented with 2 μCi/ ml Thymidine [³H] (ICN Biomedical Inc., USA). The cells were harvested after 96 hours for radioactivity count.

Rat Heart Model of Cryo-injury

After anaesthesia and intubation, rat heart was exposed through a 1.5-cm left lateral thoracotomy. Cryo-injury was induced on the free wall of the left ventricular wall using a liquid nitrogen cooled 8-mm diameter stainless probe. Cryo-injury was induced by 3 freezing cycles of 10 sec each. All animals received humane care in compliance with the institutional guidelines for animal research at NUS. A total of 21 rats were randomised into basal medium group

(n = 7), HSM group (n = 7) and HSM-VEGF₁₆₅ group (n = 7).

Myoblast Transplantation

The cells were labelled with retroviral lac-z reporter gene. Eight days after cryo-injury, 0.25 mL of medium containing 3 x 10⁶ myoblast or without cells was transplanted into rat cryo-injured heart using 27G needles. Three days before cell transplantation and until 6 weeks post-transplantation, a daily dose of 5 mg/kg/day cyclosporine was administered.

Rat Heart Function

Echocardiographic assessment of rat heart was carried out using 12 mHz probe positioned on the left anterior side of the chest. The heart was imaged in 2D mode at minimum depth settings to measure ejection factor (EF) and fractional shortening (FS).

Tissue RT-PCR Analysis of Rat Heart Post-myoblast Transplantation

Rat heart tissue was collected for RT-PCR analysis. The isolation of total RNA and RT-PCR analysis was performed as described earlier. The same primers were used for hVEGF₁₆₅. The primers for rat GAPDH were (forward) 5'-TTCTTGTGCAGTGCCAGCCT CGTC-3', (reverse) 5'-TAGGAACACGGAAGGCCATGCCAG-3'. The comparison of signals was performed using Microimage Analysis Software (Olympus, Japan).

Immunostaining of Rat Tissue for von Willebrand Factor VIII Expression

Tissue sections of rat heart were fixed in 100% acetone for 30 seconds. After blocking with 1% BSA, 1:2000 dilutions of rabbit anti-von Willebrand Factor VIII antibody was added. After 24 hours incubation at room temperature, the antigen was visualised using goat anti-mouse IgG conjugated with HRP and developed by DAB system.

Statistical Analysis

Statistical analysis was performed using SPSS (version 11.0). All data were presented as mean ± standard error mean (SEM) and analysed by analysis of variance (ANOVA). *P* < 0.05 was considered statistically significant.

Results

The purity of myoblast was >98% as assessed by desmin expression (Fig. 1a). The transduction efficiency of retroviral vector carrying lac-z reporter gene with myoblast was 75-80% (Fig. 1b). A high transduction efficiency (>98%) was achieved for VEGF₁₆₅ by a triple transduction procedure at a ratio of 1:1000PFU myoblast to Ad-VEGF₁₆₅ vector (Fig. 2a). ELISA revealed that the HSM-VEGF₁₆₅ secreted VEGF₁₆₅ up to 30 days *in vitro* with peak level at day 7 post-transduction. The peak expression level was 37 ± 3 ng/mL which was significantly higher than non-transduced myoblast (0.2 ± 0.04 ng/mL, *p* = 0.000). RT-PCR analysis of HSM-VEGF₁₆₅ was consistent with ELISA data and further confirmed high expression level of hVEGF₁₆₅ (Fig. 2b) Thymidine incorporation assay revealed that HUVEC cultured with conditioned medium from HSM-VEGF₁₆₅ incorporated significantly high level of Thymidine than any other group (*p* = 0.000).

β-gal staining revealed that myoblast successfully survived in rat heart (Fig. 3). Immunostaining for von Willebrand Factor VIII expression revealed that blood vessel density in HSM-VEGF₁₆₅ group at low power field (x200) was 17.5 ± 1.05. It was significantly higher than DMEM group 3.6 ± 0.7 (*p* = 0.000), and myoblast group 5.44 ± 0.82 (*p* = 0.000) (Fig. 4).

Echocardiograph demonstrated that EF and FS in myoblast group (93.6 ± 1.52%; *p* = 0.019, 76.0 ± 4.95%; *p* = 0.032) and HSM-VEGF₁₆₅ group (94.4 ± 2.22%; *p* = 0.012, 77.26 ± 3.25; *p* = 0.021) was significantly higher than DMEM injected group (82.7 ± 5.43%; 54.8 ± 6.44%) (Fig. 5).



Fig. 1a.

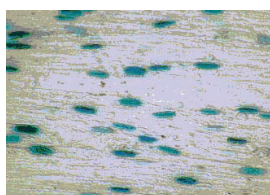


Fig. 1b.

Fig. 1. Characterisation of myoblast *in vitro*. (a) Myoblast immunostained for desmin expression. (magnification 100x) (b) Myoblast transduced with retroviral vector carrying lac-z reporter gene. (magnification 400x)

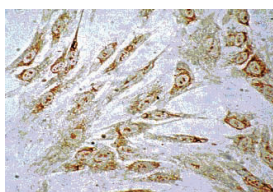


Fig. 2a.



Fig. 2b.

Fig. 2. *In vitro* characterisation of human skeletal myoblast carrying human VEGF₁₆₅. (a) Immunostaining of HSM-VEGF₁₆₅ for VEGF₁₆₅ expression. (magnification 400x) (b) RT-PCR analysis of HSM-VEGF₁₆₅ (samples 1 and 2 = myoblast, samples 3 and 4 = null-ad transduced myoblast, samples 5-12 = VEGF₁₆₅ transduced myoblast at 1, 8, 18 and 30 days post transduction).

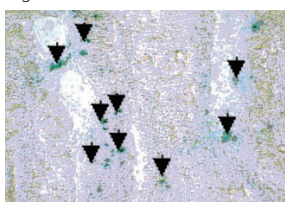


Fig. 3. Lac-z staining of myoblast transplanted rat heart (a) Myoblast located in cardiomyocytes. (magnification 200x) (Black arrow shows the presence of lac-z carrying myoblast)

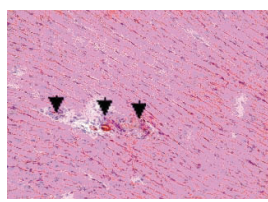


Fig. 4a.

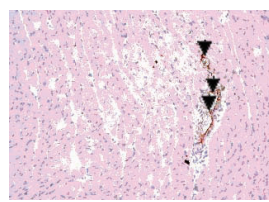


Fig. 4b.

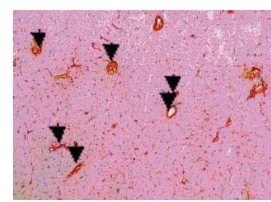


Fig. 4c.

Fig. 4. Rat cardiac tissue immunostaining vWF-VIII expression. The number of the blood vessels in HSM-VEGF₁₆₅ transplanted rat heart (c) was significantly higher than DMEM injected rat hearts (a) and myoblasts transplanted rat hearts (b). (magnification 200x) (Black arrow shows the blood vessel)

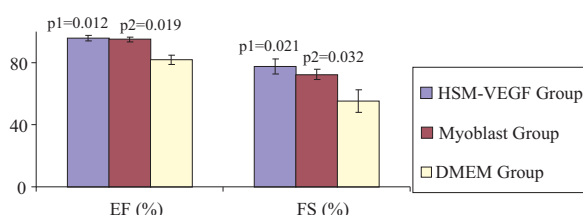


Fig. 5. Rat heart function post-myoblast transplantation. Ejection fraction (EF) in myoblast and HSM-VEGF₁₆₅ groups was significantly higher than DMEM-injected control group animals. Fractional shortening (FS) of myoblast and HSM-VEGF₁₆₅ transplanted animals was significantly higher than DMEM-injected control group (p1: HSM-VEGF₁₆₅ group vs DMEM group, p2: myoblast group vs DMEM group).

Discussion

The present study demonstrated that transplantation of human skeletal myoblasts carrying human VEGF₁₆₅ gene efficiently improved heart function in a rat heart model of myocardial infarction. The genetically engineered myoblast efficiently achieved therapeutic angiogenesis.

The efficiency of therapeutic angiogenesis using VEGF is related to its dose and delivery strategy. It has been shown that low dose for longer term administration has shown better prognosis with less side effects as compared to higher dose administration.^{6,7} Apart from the dose, route of administration significantly influences the result of angiogenesis.³ Thus, a regional and sustained level for a sustained term delivery of VEGF is necessary to achieve therapeutic angiogenesis. In the present study, we choose myoblast as a carrier of hVEGF₁₆₅ for therapeutic angiogenesis in cardiac repair. HSM-VEGF₁₆₅ efficiently secreted hVEGF₁₆₅ up to 30 days *in vitro* and *in vivo*. The transplanted HSM-VEGF₁₆₅ served as a reservoir to secrete biologically active hVEGF₁₆₅ in a localised pattern.

Myoblast was transplanted into centre and peri-infarction as a localised therapy. No angioma formation was observed at 6 weeks post transplantation. Tissue RT-PCR analysis revealed that HSM-

VEGF₁₆₅ worked as an expression vehicle and expressed hVEGF₁₆₅ in a regulated manner. We hypothesised that this novel strategy could deliver VEGF₁₆₅ in a site specific and sustained pattern.

We concluded that transplantation of human skeletal myoblast carrying human VEGF₁₆₅ gene resulted in a regional, sustained and transient expression of VEGF₁₆₅ for myocardial repair. This strategy leads to increased vascular density and improved heart function. This clinical related strategy is promising for patients suffering from myocardial infarction.

Sources of Support Funding

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Use of Traditional Medicine in an Acute Upper Respiratory Tract Infection in Singapore

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Introduction

Although upper respiratory tract infections (URTIs) are commonly seen in Singapore, the treatment strategies are very varied. They range from antihistamine,¹ antitussives,² and antibiotics;³ to non-pharmacological methods such as inhalation of steam.⁴ However, there is very little research on the use of traditional medicine in URTI. This study aims to determine the profile of the adult URTI patients in Singapore who use the traditional medicine while they consult Western trained doctors.

Materials and Methods

821 consecutive patients who attended a group of 9 polyclinics in National Healthcare Group, Singapore for acute URTI in March 2002, and fulfilled the criteria, were interviewed. 595 responded, giving the rate of 72.4%. A detailed questionnaire was used, exploring their social status, URTI symptoms and use of traditional medicine in the past, and the current types used by them.

Results

There were 190 patients who used traditional medicine (32.0%). Other subjects were taking vitamin C, cold preparations, cough mixtures and antipyretics. Only 27 subjects (4.5%) did not take any medications or supplements at all. The common traditional medicine used were “cooling tea” (104, 17.5% of total), “pipakao” (48, 8.1%), “luohan” or Arhan fruit (24, 4%), and chrysanthemum tea (20, 3.4%); all of them were of the Chinese origin. There was no significant difference in the profile of these patients in terms of gender and educational level. There were more adults above 40 years old who used these medications. Almost half of the Chinese patients used these traditional Chinese medicine (TCM) compared to the other ethnic groups (Chinese 41.8%, Malay 8.7%, Indians 10.8%, Others 23.1%; $p < 0.001$). We found that 120 patients (63.2%) were using the traditional medicine alone for about 3 days before they went to see the polyclinic doctors ($p = 0.002$). 52 of them (28.5%) used 2 or 3 traditional medicine before the polyclinic visit. Among the symptoms reported by the patients on traditional medicine, majority of them were related to throat discomfort (about 70%), cough (about 89%) and nose symptoms (about 60%). 40% of them felt feverish. Noticeably in the clinical examination, majority of these patients have temperatures less than 37°C (58.4%), and 36.3% had temperature between 37°C and 38°C.

Discussion

From this study, we noted that 32% of the subjects used traditional medicine. They could have used other medications as well, such as over-the-counter Western medicine and prescribed medicine from past doctor consultations. Only 27 participants (4.5%) did not try anything before going to see the polyclinic doctors. We could see that the traditional medicine used by the subjects were common items readily available in the local market, such as cooling tea, “pipakao” and Arhan Fruit. Most people would not resort to use expensive herbs for relieving common cold symptoms.

We have found that 35.6% of those who used traditional medicine this time used it previously, this was found to be significant. It is very likely that people tend to rely on their own experience to treat URTI on their own. We found that 120 patients (63.2%) were using the traditional medicine alone for about 3 days before they went to see the polyclinic doctors, and about one quarter tried more than one traditional medicine. It showed that a majority of the subjects would want to try their own methods of relieving their URTI symptoms before consulting a Western-trained doctor. This is particularly important in Singapore, which was affected by the Severe Acute

TABLE I: DEMOGRAPHICS AND CHARACTERISTICS OF SUBJECT PATIENTS WHO ATTENDED THE NHGP POLYCLINICS FOR URTI SYMPTOMS

Demographic	Variable	No.	%
Age (y)	21-40	232	39.8
	41-60	246	42.1
	>60	106	18.1
Ethnicity	Chinese	410	69.0
	Malay	104	17.5
	Indian	67	11.3
	Others	13	2.2
Gender	Male	318	53.5
	Female	276	46.5
Education group	Primary school and below	294	49.6
	Secondary school and vocational	189	31.9
	Preuniversity and tertiary	110	18.5
Payment of medical bills	Self	281	47.2
	Not-self	312	53.0
Smoking status	Non-smoker	470	79.3
	Current smoker	123	20.7
Regular doctor	Polyclinic	366	61.6
	Others (GP, private specialists)	228	38.4

TABLE II: CURRENT METHODS USED IN THE MANAGEMENT OF URTI

Description	No. used	% of total
Traditional medicine	190	32.0
Cooling tea	104	17.5
Pipakao	48	8.1
Rhinoceros horn	7	1.2
Ginseng root	14	2.4
Ling yang (antelope horn)	12	2.0
Luo han guo (arhan fruit)	24	4.0
Chap tiga kaki	2	0.3
Chrysanthemum tea	20	3.4
Others	13	2.2
Over-the-counter medicine	295	49.7
Vitamin C	34	5.7
Zinc supplements	2	0.3
Cold preparation	52	8.8
Multi-vitamins	11	1.9
Cough mixture	78	13.1
Pain killers/antipyretics	218	36.7
Nil	27	4.5

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TABLE III: CHARACTERISTICS OF PATIENTS ON TRADITIONAL MEDICINE

Description	Number on traditional medicine	% in traditional medicine usage	% in individual group
Gender			
Male	94	49.5	29.7 of males
Female	96	50.5	35.3
Age			
21-30 years	27	14.6	24.5
31-40	32	17.3	26.7
41-50	56	30.3	35.9
51-60	34	18.4	38.6
>60	36	19.5	34.3
Race			
Chinese	170	89.5	41.8
Malay	9	4.8	8.7
Indian	7	3.7	10.8
Others	3	1.6	23.1
Education			
Primary School and below	94	49.5	32.4
Secondary School and vocational	64	33.7	33.9
Pre-University and tertiary	32	16.8	29.4

TABLE IV: DURATION OF TRADITIONAL MEDICINE USAGE, AND REASONS TO SEE POLYCLINIC DOCTORS

Question	Traditional medicine No. (% of trad med usage)	
No of traditional medicine used by patients before seeing polyclinic doctors.	1 2 3	130 (71.4) 41 (22.5) 11 (6.0)
How many sick days before going to polyclinic?	0-3 days 4-7 days >7 days	120 (63.2) 69 (36.3) 0
How many times patient sought treatment before polyclinic?	0 time 1 time 2 times	182 (95.8) 7 (3.7) 1 (0.5)
How many Western doctors seen before polyclinic visit?	1 time 2 times	3 (1.6) 1 (0.5)
How many traditional healers before polyclinic visit?	1 time 2 times	4 (2.1) 0
Reasons to see polyclinic doctors		
Symptoms more severe		63 (33.2)
Need medication for symptoms		52 (27.4)
Symptoms last longer		17 (8.9)
Need antibiotics		0
Needed medical leave		15 (7.9)
Needed to get well faster		32 (16.8)
No improvement of symptoms		10 (5.3)

Respiratory Syndrome (SARS) in March 2003. We found that the patients might be seeking treatment from traditional sources, such as medicinal shops or traditional massagers, before seeing a Western-trained doctor or a qualified TCM physician who is trained to identify SARS symptoms and signs. By then, the patient might have unknowingly infected other people.

There were almost an equal number of male and female participants who used traditional medicine in this study. As suspected, the middle-aged to elderly groups were the ones using this method. It was not surprising that Chinese patients tend to use traditional medicine. We were unable to determine what traditional medicines were the other races using as the numbers were small. There was no significant difference in the profile of the patients in terms of educational levels as well.

Generally, there was no significant symptom that made the patients seek relief with traditional medicine, though the majority had nose symptoms, cough and phlegm. About 40% of them had fever; this suggested that patients might have taken fever seriously and would seek medical attention earlier than other nose and throat symptoms. This was shown in the clinical examination that there were very few patients with temperatures more than 38°C (7 subjects, 3.7%).

TABLE V: SYMPTOMS ASSOCIATED IN SUBJECTS

Symptom	Traditional Medicine (% of trad med usage)
Cough	170 (89.5)
Blocked nose	114 (60.6)
Runny nose	116 (61.4)
Itch at the back of nose & throat	134 (70.5)
Sneezing	114 (60.0)
Phlegm in throat	140 (73.7)
Throat clearing	132 (69.5)
Sore throat	142 (74.7)
Dry mouth	151 (79.9)
Hoarse voice	137 (72.1)
	p=0.001
Fever	76 (40.0)
Drowsiness	92 (48.7)
Dizziness	65 (34.4)
Body aches	107 (56.3)
Weakness	126 (66.3)
Shortness of breath	70 (37.0)
Abdominal pain	27 (14.2)
Nausea	33 (17.4)
Diarrhoea	27 (14.2)
Current Smoking	27 (14.2)

There is no significant association between the symptoms and taking traditional medicine, except for hoarse voice (p=0.001, Chi-Square, OR=1.85, 95% CI 1.27 to 2.69)

TABLE VI: SIGNS ASSOCIATED IN THE SUBJECTS

Clinical examination	Traditional medicine No. (% of total)
Body temperature (<37.0 (degree C)	111 (58.4)
37.0-38.0	69 (36.3)
>38.0	7 (3.7)
Nasal discharge	102 (53.7)
Throat injection	131 (68.9)
Tonsils enlarged/exudates 10	10 (5.3)

Conclusion

This study is significant in identifying the pattern of user of traditional medicine in the self-treatment of URTI. The user is likely to be a middle-aged male or female; usually a Chinese who uses TCM. He or she may have varying educational level, but will rely on his or her past experience to try these medications for a few days (<7 days) before seeing a Western-trained doctor. But when the person has fever, he or she is more likely to see a doctor earlier.

This information is useful for the primary care physician in that there are known reports of drug interactions and side effects with the use of the TCM.⁵ It is difficult to identify all the components of these medications, as there are the concoctions of various herbs and fruits brewed together. The doctor will need to find out from his local patients routinely, what other medication he or she is on, so as to identify any possible allergies with the TCM.

When the patient turns up at the clinic for URTI symptoms, he is likely to have taken some traditional medicine. Therefore, if the symptoms persist, he would expect the doctor to act on it more thoroughly such as the need for blood test (full blood count) or for prescription of antibiotics. The primary care physician would need to assess each case individually to determine the need for more laboratory investigations and discuss with the patient on the usage antibiotics in URTI.

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Fever Phobia in a Primary Healthcare Setting: A Singapore Perspective

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Introduction

Fever in children is a commonly seen problem in the primary healthcare setting but one which often alarms parents.¹ Fever phobia can lead to potentially aggressive and inappropriate management of a febrile child.²⁻⁴ Our study seeks to examine the prevalence of fever phobia defined as worries regarding fever, amongst parents visiting the polyclinic as well as their ways of monitoring temperature, past experiences with healthcare practices, traditional practices as well as resources for fever management in children.

Materials and methods

Using a standard questionnaire, modified from that used by Crocetti et al,⁴ a cross-sectional survey of 557 consecutive parents was conducted at the Ang Mo Kio Polyclinic in May 2002. The inclusion criteria included parents of children between 3 months old and 5 years old who were brought to the polyclinic for a well- or sick-visit. A well visit was defined as health maintenance visits for purposes such as immunisation or developmental assessment. A sick visit was defined as acute and follow-up visits for management of medical conditions such as upper respiratory tract infections and gastroenteritis. The questions elicited information including the following: the definition of fever, concerns about fever, frequency of temperature monitoring, fever management including home fever reduction methods, and parental recall of treatment that their children had received during health care visits for fever, observed health care practices for fever during visits to their family physicians. The questionnaire was administered in the three major spoken languages – English, Mandarin and Malay. The English questionnaire was translated into Mandarin and Malay, then back translated to English by bilingual speakers to ensure a high accuracy in the translation of the questionnaire items. Training was provided to standardise the way in which the questions were asked in order to minimise interviewer bias. Written informed consent was taken from all participants and the study was approved by the Research and Ethics Committee.

Results

Of the 600 eligible parents who were approached, 557 agreed to participate giving a response rate of 92.8%. In terms of ethnicity, 70.4% were Chinese, 16.7% were Malay, 10.2% were Indian and 2.7% belonged to other ethnic groups. In terms of age of the child, 81.1% were between 3 and 36 months and 18.9% older than 36 months. Mothers constituted 78.1%, fathers 16.5% and both parents 5.4% of the respondents

Amongst the parents, 271 (48.6%) considered a temperature equal to or less than 37.8°C (100°F) to be a high fever and 24 (4.3%) believed that the temperature could rise to 43.3°C (110°F) or above if left untreated. Two hundred and eighty-one parents (50.4%) were very worried about fever. Specifically, 510 (91.5%) were worried that fever could possibly cause harmful effects such as brain damage (n = 383, 68.8%), seizure (n = 78, 14.1%) and death (n = 19, 3.4%). Almost half (49.9%) of the parents worried more if the child had a high temperature than if their child looked sick during a febrile illness. Nearly three-quarters (70.5 %) of the parents would check their child's temperature every hour or less when their child had a

fever. In terms of antipyretic use, 138 (24.8%) of parents would administer paracetamol for temperatures equal or less than 37.8°C (100°F) and 478 (85.8%) parents would give antipyretics before the temperature reached 38.9°C (102°F). In terms of fever reduction methods, about the same proportion of parents would give paracetamol either in tablet (38.7%) or liquid form (40.3%) at up to four hourly intervals. In terms of fever reduction technique, 449 (80.6%) parents used sponging including 50 (9.0%) who would initiate sponging for temperatures equal to or less than 37.8°C (100°F).

Forty-one (7.4%) of them would consult the traditional medical practitioner and 62 (11.1%) administer traditional medicine concomitantly with the polyclinic consultation. Traditional practices adopted by parents when their child had fever included showering the child (55.1%), switching off the air conditioner (27%), switching off the fan (11.8%) and wrapping or bundling their child (5.8%). We found that 41 (7.4%) parents would consult traditional doctors as well as Western doctors for their child's fever, while 62 (11.1%) would give traditional herbal remedies to their children during a febrile illness. Of note, 25 (4.5%) parents would give talisman water to their febrile child to drink, 8 (1.4%) would put oil on the child's fontanelle and 7 (1.3%) would put special herbs on the child's hand and feet in an attempt to cool the child.

The most important perceived source of fever knowledge was the medical staff, namely doctors and nurses.

Discussion

There were differences in the nature of the perceived harmful effects between the parents in our current study and that in the Western population. A large proportion (68.8%) of our parents believed that brain damage might be a harmful consequence. This is lower than the findings in previous studies which reported that between 21% and 45% of parents were concerned that brain damage may be an untoward consequence of fever.^{2,4} However, death as a possible perceived harmful consequence was found to be lower in this study (3.2%) compared to that in the earlier two studies which found rates of between 8% and 14% in the parents. In the Asian culture, great importance and premium are commonly placed on educational attainment. This may lead parents to worry more greatly about the effect of the fever on the brain of the child and hence the future consequences such as academic potential rather than death per se although this needs to be further evaluated.

Parents need to be educated about identifying fever as a symptom of an underlying disease rather than the disease itself. In our study, almost half of our parents felt that the level of fever rather than the clinical toxicity of a child was a better indicator in correlating with the likelihood of any serious infection in the child. The clinical features of a sick and toxic child include lethargy, low hydration status, hypoventilation or hyperventilation and cyanosis. These features are just as important for the parents to recognise than to solely focus and depend on the level of fever.⁵ The excessive focus on the level of fever may be translated into excessive temperature monitoring and usage of medication as found in this study. Although the majority of the parents used sponging as a temperature reduction

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method, a smaller proportion of parents would initiate sponging or administer an antipyretic medication at a temperature of equal to or less than 37.8°C (100°F) which was comparable with the findings of the study by Crocetti et al.⁴ However, our parents were more conservative in the dosing frequency and interval of administration of the antipyretic medications. A larger proportion of parents in this study would leave the fans and air-conditioners on perhaps in an attempt to reduce the fever in the child. On the other hand, the practices of not bathing the child, switching off the fan and the air conditioners in other parents could also be attempts to prevent shivering which is commonly observed and may ultimately raise further the temperature of the febrile child. Of note, was the promotion of the ingestion of talisman water as a fever reduction method in a small proportion of the parents. A number of parents would also seek traditional medical practitioners for consultation and resort to taking traditional medicine to manage the fever. The effects of these traditional practices as well as that of the traditional medicines are often not studied and benefits not fully appreciated. Further studies are thus needed to explore the modes of action and possible benefits of these traditional medicines and practices. It also underlines an important need to understand the cultural beliefs and biases of both the parents as well as the clinician in the management of the febrile

child with implications in areas of establishment of therapeutic rapport and alliance. This will lead to a more effective management of the ailment and yet incorporating the explanatory model of the parents

In conclusion, fever phobia is common amongst the parents visiting the polyclinic. The beliefs, practices and perceived source of knowledge for management of the febrile child lend itself to interventions such as better parent education with the potential to reduce needless consultations.

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Best Oral Presentation Award Finalist – General Practice

Revisiting Upper Respiratory Tract Infection (URTI) in Adults in the Light of the Current Severe Acute Respiratory Syndrome (SARS) Situation in Singapore

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Introduction

Upper respiratory tract infection (URTI) is the most common reason for attendance in primary care practice in Singapore, constituting 30% of all attendances seen at primary medical care clinics in year 2001 (unpublished data – Emmanuel S, et al. Survey of primary medical care in Singapore 2001).

In the advent of the new atypical pneumonia called severe acute respiratory syndrome (SARS), whose early phase of the disease can mimic symptoms of URTI,^{3,4} primary care physicians have been thrown into a quandary as to what is simple URTI and what is not. Considering that the consequence of missing an early SARS case has significant morbid and epidemiological implications, it is not uncommon for primary physicians to find the management of any URTI case that walks into the consult a tad more stressful than before. Short of a better characterisation of the clinical presentation of SARS, or better still, a bedside diagnostic kit, the other way primary physicians can cope with this challenging situation is to know its differential diagnosis, URTI, viral fever and dengue fever very well.

A lot of work in URTI has been done on the health seeking behaviour of patients, antibiotic use, therapeutics, prevention and epidemiology in URTI,⁵⁻¹⁴ but not on its symptoms. Barret et al

studied the symptoms and its severity in 2002 as part of a validation of the Wisconsin Upper Respiratory Symptom Survey.¹⁶

The aim of the study was to characterise URTI using detailed symptoms, signs, investigations and outcomes in adult patients who attend a cluster of 9 primary health centres in Singapore and then to compare this with the reported symptomatology of SARS.

Materials and Methods

Subjects

We conducted this prospective study in all 9 clinics belonging to the National Healthcare Group Polyclinics (NHGP). We recruited patients sequentially from each clinic, having first determined the order in a random fashion. Approval to conduct the study was obtained from the National Healthcare Group Ethics Committee.

The study population included 594 consecutive patients who presented with complaints of respiratory tract symptoms from February to April 2002. Inclusion criteria were all patients who were Singaporean residents, aged 21 years and above, who complained of any one of the following, namely cough, nasal (sneezing, runny nose, blocked nose) or throat (sore throat, throat clearing, phlegm in throat) symptoms of <7 days' duration. Patients were excluded if they had history of chronic respiratory problems such as allergic

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rhinitis, chronic sinusitis, asthma or chronic obstructive pulmonary disease, were currently on treatment for tuberculosis, immunocompromised, on long-term antibiotic use, recent antibiotic use in the past 2 weeks, recent travel or hospitalisation, pregnant or currently breastfeeding. Out of a total of 821 eligible patients, 595 gave signed informed consent to participate in the study

Data Collection

We collected data by interview using a structured questionnaire, clinical examination using a standard recording form, blood sample for total white and differential count, throat swab for bacterial culture, and nasal swab for virus identification. Patients were followed-up by phone interviews till resolution of symptoms.

Sections in the questionnaire included questions on the severity of each symptom from a list of 23 symptoms. All interviews and biological sample collection were conducted by the authors and trained nurses. Resident doctors in each of the clinics, who have been briefed on the study protocol, conducted the physical examination and recorded the findings in structured recording forms.

We followed each patient up 1 week later by a telephone call. Patients were asked if there were any change in severity of each symptom in the list, whether there were new symptoms, any side effects of treatment experienced, further medical help sought, and any complications diagnosed. For patients who have not recovered by the first week, second and subsequent phone calls were made till resolution of all symptoms.

Full Blood Count

Full blood count was performed using the Cell-Dyn Haematology Analyzer (Abbott Laboratories).

Bacterial Culture

All bacterial culture and virus identification were performed at the laboratory of one of the authors (RTP Lin).

The potential pathogens looked for were β -haemolytic streptococci of Lancefield groups A, C and G; and predominant heavy growth of *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* using standard methods.¹⁶

Immunofluorescence Microscopy for Respiratory Viruses

We used flexible wire swabs (Copan, Italy) to obtain specimens from patients' nasopharynx and transported in the virus transport medium. The specimens were processed for immunofluorescence for influenza A, influenza B, parainfluenza 1, parainfluenza 2, parainfluenza 3 viruses, respiratory syncytial virus and adenovirus and rhino virus.¹⁷⁻²¹

Results

Descriptive Profile of Patients

There were 318 males (53.5%) and 276 females (46.5%). The mean age was 45.4 years (standard deviation [SD]: ± 14.9), and median 45.0 years (range 21-88). The ethnic distribution was Chinese 69.1%, Malay 17.5%, Indian 11.1% and Others 2.2%. 69.8% were non-smokers, 20.7% current smokers and 9.4% ex-smokers.

Symptoms

The most common symptom was cough (88%), followed by dry mouth (76%). Phlegm in the throat, throat clearing, sore throat, runny nose, itch at the back of throat, hoarse voice, sneezing and block nose occurred around the same frequency (62-73%)

More than half the patients also reported body aches, weakness, difficulty sleeping and drowsiness. Whilst 39% reported having fever, only 11% were detected to have fever at physical examination. 32% reported shortness of breath and 26% reported chest tightness. However, only 1.3% (8) patients were found to have abnormal

auscultatory findings. Up to 15% of the patients reported some form of gastrointestinal symptoms, e.g., nausea, abdominal pain, diarrhoea and vomiting.

In terms of symptom resolution, cough, phlegm in the throat, runny nose and drowsiness tended not to resolve as fast as symptoms like difficulty sleeping, weakness, fever, chest tightness, shortness of breath and gastrointestinal symptoms.

Physical Examination

78% of patients who attended for URTI symptoms had throat injection. Nasal discharge was observed in 53% of patients. Tonsillar enlargement and exudates and cervical lymphadenopathy occurred less than 8% of the cases.

Laboratory Findings

Total white count and the lymphocyte count were predominantly within normal range. More than half of the polymorphonuclear leukocyte count were less than 60%.

A viral agent was detected in 25% (147) of the cases, of which 133 were rhinoviruses. Only 2% (11) of the cases had a bacteria isolated. 3 cases had both viral and bacterial agents isolated. There were no cases of Group A beta *Haemolytic streptococcus* isolated. 73% of the cases had growth of normal flora.

Outcome Parameters

Only 30% of the patients had less than 9 symptoms at consultation, with 31% reporting more than 13 symptoms. The mean number of symptoms was 11. However, by the first review, 66% of the patients had <9 symptoms. 49% of the patients recovered fully after 7 days. 7% were still unwell after 14 days. And longest duration of symptoms was 33 days. Less than 4% had either facial pain or ear pain but 18% reported some complication of medication, with sedation as the most common side effect affecting 14% of the patients. 9% (52) patients consulted again after the index consultation for the same episode of URTI.

Comparison with the Reported Symptoms of SARS (Table I)

Cough, body aches and shortness of breath occurred with almost the same frequency as that of SARS patients in both the Hong Kong and Canadian clusters^{3,4} as well as the cluster of 135 patients from Singapore (Leo YS, et al. CDC Singapore. Singapore SARS task force).

Fever was more than twice as common in SARS; rhinorrhoea was more than 6 times and sore throat more than twice as common in URTI.

TABLE I: SYMPTOMS OF SARS AS REPORTED BY VARIOUS STUDIES COMPARED TO THE NHGP URTI STUDY

Symptom	Singapore SARS n = 135 (Unpublished data)	Hong Kong SARS n = 138 <i>N Engl J Med</i> April 2003 ⁴	Canada SARS n = 10 <i>N Engl J Med</i> March 2003 ⁵	NHGP URTI Study n = 594
*Fever	100%	100%	100%	39%
Cough	70%	57%	100%	88%
Myalgia	51%	61%	20%	59%
Dyspnoea	34%	60%	80%	32%
Nausea	31%	20%	10%	15%
*Sore throat	26%	23%	30%	70%
Diarrhoea	22%	20%	50%	10%
Malaise	19%	70%	70%	59%
Headache	18%	-	30%	-
*Rhinorrhoea	5%	10%	-	67%

Discussion

Primary health physicians may find symptoms, such as cough, body aches and shortness of breath, less useful in the diagnosis of early SARS, in view of substantial overlap in frequency of such symptoms. Fever, sorethroat and runny nose occur consistently in different frequencies between the 2 diseases and may help in differentiating them, bearing in mind that the knowledge of the clinical presentation of SARS is still evolving.

The description of the natural history of symptoms will also be useful in educating physicians, patients and employers with regards to realistic expectation of resolution of symptoms. During the current SARS situation, primary physicians have been seeing increased number of repeat attendances because of the failure of symptoms to resolve promptly and the understandable anxiety of the patients who are still symptomatic.

The number of complications from URTI was negligible except for the side effects caused by medication. Over medicalizing this self-limited disease should also be avoided.

Conclusion

In a group of 594 adults attending for URTI in NHGP, the most common symptom is cough, followed by dry mouth and phlegm in the throat. Each patient had experienced an average of 11 symptoms. The most common signs are throat injection and nasal discharge. Viral cause was 12 times more common than bacterial. The disease is self-limiting and the most common complication of URTI was side effect due to medications.

Symptoms such as fever, runny nose and sore throat may be more useful in differentiating early SARS from URTI than cough, shortness of breath and body aches.

Acknowledgement

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NF- κ B Plays a Protective role in Nitric Oxide-induced Neuronal Apoptosis

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Introduction

The physiological signalling molecule, nitric oxide (NO), plays an important role in numerous functions in cells. However, excessive excitatory neurotransmission can result in its over-production, and accumulating evidence suggests a role of NO in neuronal apoptosis, which contributes to many neurological disorders, including stroke, seizure and Alzheimer's disease.

NF- κ B is a ubiquitous transcription factor that participates in many neuronal processes, including synaptic transmission, neural development and neurodegenerative diseases. Recent data revealed that NF- κ B can have anti-apoptotic or pro-apoptotic roles under different circumstances. In NO-induced neuronal apoptosis, NF- κ B was activated, however, its significance was still unclear. Here, we demonstrated that NF- κ B plays a protective role against NO-induced apoptosis in human neuroblastoma cells. The protective mechanism may involve either a delay or inhibition of cytochrome C release from the mitochondria into the cytosol.

Materials and Methods

Cell culture: Human SHEP-1 neuroblastoma cells were maintained in Dulbecco's modified Eagles's medium containing 10% fetal calf serum, 100 units/mL penicillin, and 100 ug/mL streptomycin. LipofectAMINE was used in all transfections, employing the protocol provided by Life Technologies, Inc. Transformed clones were selected and maintained with 500 ug/mL G418.

Cell death assay: Different concentrations of the NO donor, sodium nitroprusside (SNP) was added to the medium for 16 to 24 hours. Cell death was measured by crystal violet staining.

Preparation of cytosolic and mitochondrial proteins: Wildtype and stably transformed SHEP-1 cells were harvested and washed in ice-cold phosphate-buffered saline. To isolate cytosolic proteins, cells were homogenised in a dounce homogeniser in cold buffer-1 (10mM Tris-HCl pH=7.5, 0.3M sucrose, complete protease inhibitor cocktail). After centrifugation at 10,000xg for 60 minutes at 4°C, the supernatant was collected and designated as the cytosolic protein fraction. The cell pellet was resuspended in cold Buffer-2 (10 mM Tris-HCl pH = 7.5, 1% Triton X-100, 150 mM NaCl, complete protease inhibitor cocktail) and sonicated using a Ultra sonicator. Following centrifugation at 10,000xg for 30 minutes at 4°C, the supernatant was collected and designated as the mitochondrial protein fraction.

Western blot: Twenty micrograms of cytosol and mitochondria proteins were electrophoresed in 14% polyacrylamide gels and transferred to nitrocellulose membranes. Immunoblotting was performed with antibodies diluted in phosphate-buffered saline (PBS) containing 0.2% Tween 20 and 5% bovine serum albumin (BSA). After several washing steps, the membrane was probed with a horseradish peroxidase-conjugated secondary antiserum. This was followed by further washing and chemiluminescence detection of antibody binding.

Immunohistochemistry: SHEP-1 cells were seeded on chamber slides and treated with insulin growth factor- I (IGF-1) for 1 to 6 hours. This was followed by fixation of the cells with 1%

paraformaldehyde and blocking with 5% BSA. Fixed cells were incubated with an antibody against NF- κ B (P65) for 1 hour. After several washes with PBS, cells were incubated with FITC conjugated secondary antibody. The staining results were visualised using a fluorescence microscope.

Results

NF- κ B is activated by NO and plays a protective role in neuronal apoptosis induced by NO

The generation of NO in cells can be mimicked with chemical donors of NO. The NO donor, SNP, releases NO intracellularly, which induces apoptosis of neuroblastoma cells partly via oxidative stress. Our previous work has demonstrated that 0.5-2 mM SNP can effectively kill neuroblastoma cells after 8 hours of treatment. The transcription factor NF- κ B is activated prior to cell death, at 1 to 4 hours post treatment. To investigate the significance of NF- κ B activation, we generated SHEP-1 cell lines expressing I- κ B α M, a dominant negative mutant of I- κ B α . I- κ B α M prevents the release of NF- κ B from its associated complex in the cytoplasm and therefore inhibits NF- κ B translocation to the nucleus. With NF- κ B activation blocked, I- κ B α M cells show markedly increased sensitivity to apoptosis induced by SNP. Similar results were obtained with another I- κ B α M expressing clone. Therefore, our data indicates that NO stimulated NF- κ B activation protects SHEP-1 cells from apoptosis.

NF- κ B may help to stabilise the mitochondria

NO has been demonstrated to induce cytochrome C release, which in turn trigger a series of events that ultimately leads to apoptosis. To analyse the mechanism by which NF- κ B protects SHEP-1 cells against NO-induced apoptosis, we measured the release of cytochrome C from the mitochondria. Cytosolic proteins were isolated from wildtype and I- κ B α M cells treated with 1mM SNP. Western blot was performed using a cytochrome C specific antibody. Interestingly, the results reveal cytochrome C to be clearly present in the cytosol of I- κ B α M cells, but in significantly lesser amounts in wildtype cells. This suggests NF- κ B activation in wildtype cells can prevent cytochrome C release from mitochondria. Since cytochrome C release plays an important role in the execution phase of apoptosis, NF- κ B may, via blocking cytochrome C release, protects SHEP-1 cells from cell death.

NF- κ B is required for neuronal protection by insulin-like growth factor I

To translate our results into therapeutic application, a search was done for factors with the potential of activating NF- κ B and at the same time, protect neurons from apoptosis. IGF-1 has been shown to be capable of causing NF- κ B activation, and to protect against cell death induced by various apoptotic stimuli. To determine whether IGF-1 can activate NF- κ B and protect SHEP-1 cells from NO-induced apoptosis, NF- κ B activation was measured by nuclear translocation of its p65 subunit. Consistent with our hypothesis, IGF-1 prevented cell death caused by NO, and we observed p65 immunofluorescence in the nuclei of IGF-1 treated SHEP-1 cells. In contrast, IGF-1 loses its protective effects in I- κ B α M cells where the

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activity of NF- κ B was blocked. These results demonstrated that IGF-1 can protect neurons from NO-induced apoptosis and this neuroprotection is dependent on NF- κ B activation.

Conclusion

Our study demonstrated a central role of NF- κ B in protecting neurons against the oxidative stress initiated by NO. NF- κ B is also

required for the neuroprotection mediated by IGF-1 application. The mechanism probably involves NF- κ B activation of a signalling pathway that stabilises the mitochondria, and thus prevents cytochrome C release into the cytosol. The results we obtain will be useful in helping to design a therapeutic strategy for various neurological disorders in the future.

Best Oral Presentation Award Finalist – Medicine/Paediatrics

Molecular Monitoring of Minimal Residual Disease in Childhood Acute Lymphoblastic Leukaemia Using Antigen Receptor Gene Rearrangements is Highly Feasible for Disease Stratification and Prognostication

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Introduction

Acute lymphoblastic leukaemia (ALL) is the most common form of childhood malignancy in Singapore. Despite cure rates of over 60%, relapse remains the most important cause of death. Treatment intensification reduces the risk of relapse but increases toxicity. Further improvement in cure can only be achieved through careful tailoring of the intensity of treatment to fit the patient's risk of relapse in order to achieve the optimal risk-benefit ratio.

To accomplish this, we need to accurately predict each patient's risk of relapse because the best chance for a cure is to deliver the correct intensity of therapy at initial diagnosis. Unfortunately, conventional risk stratification is based on presenting leukocyte count and age, which are imperfect predictors of outcome, giving risk-stratified therapy a low success rate. The patient's early response to therapy is the best indicator of treatment outcome as it reflects the intrinsic sensitivity of leukaemic blasts to therapy.¹⁻³ Conventional microscopy is subjective, with a low detection sensitivity of only 5 leukaemic cells in a background of 100 normal cells, below which patients are deemed to be in complete clinical remission. Leukaemic blasts below that limit are termed 'minimal residual disease' (MRD).^{2,4} Using new molecular techniques, we are able to detect and accurately quantitate MRD levels as low as 10⁻⁵, a 3-log improvement over conventional morphology.^{1,3,5,6} This provides an unprecedented ability to closely track a patient's response to therapy.

We proved previously that early response to therapy, as determined by MRD at 1 month of therapy, is a statistically significant predictive factor (Fig. 1), using Southern blotting and immunoglobulin heavy chain (IgH) rearrangements.⁷ ALL arises from clones of a single lymphoblast, thus we utilised the unique junctional regions resulting from random insertion and deletion of nucleotides.^{8,9} Southern blotting is hampered by its labour-intensiveness, and we were able to find an MRD marker for only 60% of precursor-B ALL patients, severely limiting its widespread use.

Timeliness in delivery of a patient's risk status for treatment modification is necessary, and the ability to use it on the majority of patients will determine the viability of MRD as an important prognostic factor. This study reports our turnover time for MRD reports and their applicability. The new real-time quantitative polymerase chain reaction (RQ-PCR) technology measures the product formed as the reaction progresses, removing laborious post-PCR Southern blotting. This reduces the risk of contamination and eliminates the use of radioactive reagents, making it a more rapid, more accurate and safer platform.⁹ To increase the number of informative MRD markers obtained, we investigated the feasibility of using other antigen receptors like immunoglobulin kappa deleting element (IgKde), T-cell receptor (TCR) gamma and delta gene rearrangements as targets for MRD monitoring.

Materials and Methods

Patients and Cell Samples

All newly diagnosed patients (n = 48) enrolled in the Malaysia-Singapore (MA-SPORE) ALL-2002 clinical trial and 6 relapse patients were included in this study (Table I). Week 5 samples were available from 34 patients; week 12 samples were available from 16 patients.

Detection of Gene Rearrangements

PCR amplification for IgH, IgK and TCR was carried out using primers and conditions as described previously.¹⁰ Eleven separate PCRs and heteroduplex analysis were performed for each patient. The PCR products were electrophoresed on a 6% non-denaturing polyacrylamide gel. Distinct bands were excised, precipitated, sequenced and compared against human germline sequences (<http://www.ncbi.nlm.gov/BLAST/>).

RQ-PCR

Patient-specific primers were designed from the unique junctional regions and paired with a germline Taqman reverse probe.¹¹⁻¹³

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TABLE I: PATIENT CHARACTERISTICS

Total number	54
<i>De novo</i> ALL	48
Relapse ALL	6
Precursor-B ALL	51
T-lineage ALL	3
Mean age (range)	6 years 1 month (7 months to 14 years 10 months)

TABLE II: FREQUENCY OF GENE REARRANGEMENTS

	IgH	TCRG	TCRD	IgK
Pre-B ALL (n=51)	89.8% (n=46)	77.5% (n=39)	63.2% (n=32)	61.2% (n=31)
Oligoclonal	63.3%	53.1%	ND ^b	40%
T-cell ALL (n=3)	ND ^a	66.7% (n=2)	100% (n=3)	ND ^a
Overall	89.8% (46/51)	75.9% (41/54)	64.8% (35/54)	61.2% (31/51)

^a ND, not done, as cross-lineage gene rearrangements are rare in IgH and IgK.^{9,15}

^b Monoclonality analysis not done as only one panel screened.

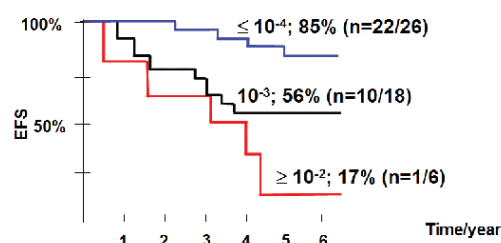


Fig. 1. MRD levels at 1 month of induction therapy is highly predictive of eventual outcome ($p < 0.05$).⁷

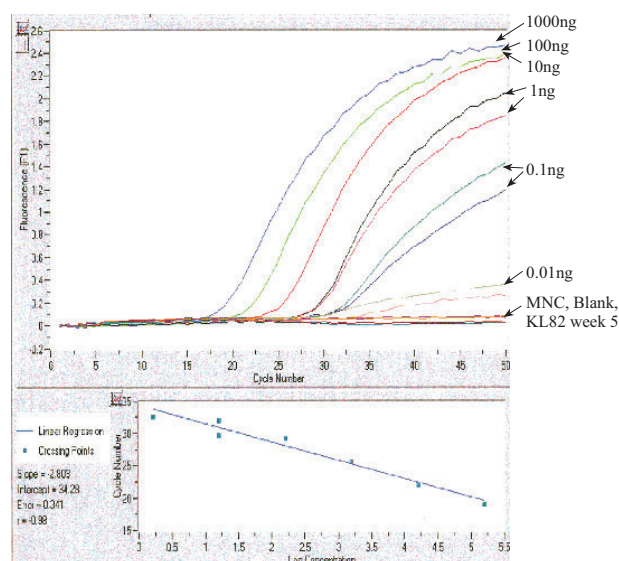


Fig. 2. A typical standard curve and week 5 sample of patient KL82. Standard curves were constructed using 10-fold dilutions of the patient's diagnostic marrow into mononuclear cells.

Diagnostic DNA samples were serially diluted 10-fold in pooled normal MNC to simulate the conditions of a remission marrow and standard curves constructed (Fig. 2). Water and MNC were used as negative controls, and the quality and quantity of the patients' DNA normalised using the albumin gene.^{12,13}

End-point Measurements

Reports for provisional and final risk group stratifications must be ready by weeks 10 and 14 respectively for marrows done on weeks

5 and 12 to enable changes in treatment protocol. Our stratification method requires at least one marker of sensitivity of 10^{-4} .

Results

Gene Rearrangements

IgH gene rearrangements have the highest frequency but suffer from the highest rate of oligoclonality, while IgK rearrangements have the most monoclones (Table II). All patients had at least one gene rearrangement using our panel of markers, with 94.4% (51/54) exhibiting 2 or more different gene rearrangements.

As at least one marker of sensitivity 10^{-4} is required, 53 of the 54 patients (98.1%; 47/48 newly diagnosed, 6/6 relapse) can be stratified according to our protocol. The levels of sensitivity and reproducibility achieved by markers from different gene rearrangements (chi-square test) showed no significant difference ($p > 0.05$). MRD reports were delivered in time for review of treatment for all patients who completed week 5 and week 12 therapy.

Risk Group Stratification

MA-SPORE ALL-2002 protocol stratifies patients at week 5 and week 12 (Fig. 3). Of the 34 remission marrows obtained at week 5, 18 (52.9%) were classified as standard risk, 12 (35.3%) as intermediate risk and 4 (11.8%) as high risk. All 4 high-risk patients were stratified thus because of the presence of high-risk oncogene fusions. At week 12, no additional patients were upgraded to a higher risk group.

To study the concordance between MRD levels determined by 2 different markers, 32 marrows from 16 patients were compared. 90.6% (13/16) were stratified into the same risk group, with only 3 discordants. Patient KL32 was initially stratified as intermediate risk based on the IgH marker which failed to reach a sensitivity of 10^{-4} , but his TCRG marker showed clearance below 10^{-4} , indicating standard risk. For patient KL76, the MRD between the two markers

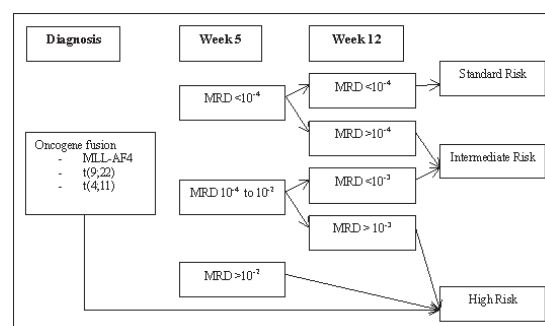


Fig. 3. Stratification of patients under MA-SPORE ALL-2003.

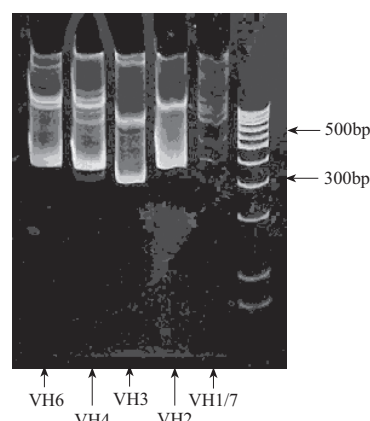


Fig. 4. Oligoclonal IgH gene rearrangements for patient D212.

TABLE III: SENSITIVITY OF THE DIFFERENT TYPES OF GENE REARRANGEMENTS USING RQ-PCR

Type of gene rearrangement	Limit of detection	Maximal sensitivity ^a
IgH (n = 29)	<10 ⁻⁴	89.7% (26/29)
TCRG (n = 10)	<10 ⁻⁴	80% (8/10)
TCRD (n = 6)	<10 ⁻⁴	100%
IgK (n = 3)	<10 ⁻⁴	66.7% (2/3)

^a The maximal level of sensitivity was defined as the maximal dilution with at least 1 replicate amplifying.¹⁷ When non-specific amplification was observed in MNC, only amplification at least 3 cycles from that of MNC was considered.

differed by one-log, which is within the standard error for MRD,¹⁴ but this difference resulted in different risk group classifications. Finally, clonal evolution caused the IgH markers of patient D212 to give false-negative results.

Discussion

We previously reported that MRD using a single IgH marker is an exceptionally powerful predictor of clinical outcome ($p = 0.03$).⁷ However, only 60% of patients could be stratified, limiting its applicability. By expanding our screening to include IgK and TCR gene rearrangements, we were able to substantially increase the percentage of patients for whom an informative marker could be obtained, from 60% to 98% ($p < 0.05$), including previously excluded T-cell ALL patients. The distribution of risk groups was similar to our previous study ($p > 0.05$).

This is an ongoing study where our protocol is to screen for a marker from IgH first, followed by TCR and IgK. Hence, of the 48 markers used for stratification, 60% were from IgH, compared with 20% or less for the other gene rearrangements (Table III). However, the highest rate of oligoclonality was also observed in IgH (63.3% vs 53.1% vs 40% for IgH, TCRG and IgK, respectively). Oligoclonal gene rearrangements are more unstable and prone to secondary rearrangement, possibly leading to false-negative MRD results.^{12,15} This was illustrated by patient D212, who exhibited IgH oligoclonality (Fig. 4), and clonal evolution occurred where 2 IgH markers were lost by week 5, giving a false-negative MRD level. Confirmation by a TCRG marker, however, detected MRD at intermediate risk level.

The AIEOP-BFM-ALL 2000 study requires 2 markers of sensitivity at least 10⁻⁴ for risk group stratification to avoid inaccurate results due to clonal evolution. Unfortunately, this stringent requirement makes it possible for the group to deliver results on time for only 67% of patients. Our study differs from the AIEOP-BFM group in that we require a minimum of 1 stable marker for MRD monitoring.

It has been widely reported that the stability of MRD targets is inversely proportional to oligoclonality, particularly for IgH.¹⁶ As we continue to screen for markers, we will prospectively explore if 1 marker is comparable to a combination of 2 markers as long as care is taken to avoid clonal evolution. If we can prove the validity of this simplified method, which is applicable to 98% of patients compared to only 67% under the AIEOP-BFM protocol, it will greatly improve the widespread application of our stratification protocol.

By using RQ-PCR, we were able to reach very high levels of sensitivity of 10⁻⁴ or more for 98% of patients, a considerable improvement over conventional techniques. As there is no significant difference between the different antigen receptor markers in terms of limit of detection and reproducibility, IgH, IgK δ , TCR gamma and delta gene rearrangements are all suitable targets for molecular monitoring of MRD.

Conclusion

Conventional risk stratification uses presenting leukocyte count and age which are imperfect risk factors, limiting its usefulness. We showed that molecular monitoring of MRD in childhood ALL is

feasible and can be applied to 98% of patients in a timely manner. With its high predictive value, MRD measurement could replace conventional risk stratification methods. Using RQ-PCR, precise quantitative monitoring of MRD with antigen receptor gene rearrangements is both sensitive (up to 3-log improvement) and highly reproducible. We have shown that in 98% of childhood ALL patients, this method can be used to tailor therapy to each child, enabling a reduction in both cost of therapy and treatment-related toxicity while maintaining an optimal anti-leukaemic effect.

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Clinical Features and Predictors for Mortality in a Designated National SARS ICU in Singapore

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Introduction

Severe acute respiratory syndrome (SARS) is a new disease attributed to a novel coronavirus (SARS-CoV).^{1,2} It began in November 2002 in Guangdong Province in southern China.³ The World Health Organisation (WHO) issued a global alert on this contagious disease on 12 March 2003. In Singapore, the first index case was hospitalised on 1 March 2003, which subsequently led to an outbreak of 206 probable SARS patients.⁴

On 22 March 2003, the Ministry of Health (Singapore) designated Tan Tock Seng Hospital (TTSH) as the only hospital for isolation and management of all SARS patients.⁵ In other countries, e.g., Hong Kong and Canada, SARS patients were managed in several hospitals.⁶⁻⁹ In TTSH, we have the unique opportunity of reviewing the clinical features and outcomes of critically ill probable SARS patients in a national designated SARS intensive care unit (ICU).

Materials and Methods

From 1 March to 9 June 2003, 206 probable SARS patients were diagnosed in Singapore. Of the 7 probable SARS patients that were not managed in TTSH, 2 ICU patients were too ill for transfer, 2 were diagnosed by autopsy in out-of-hospital deaths, 1 died in the general ward in another hospital and 2 were discharged from a third hospital. Forty-six (23.1%) of 199 patients treated in TTSH required ICU admission. We retrospectively reviewed all the 46 probable SARS patients admitted to TTSH's ICU during this study period.

Probable SARS patients were identified using WHO's criteria current at that time.¹⁰ Patients were referred to the ICU if they developed progressive respiratory failure or required at least 50% O₂ in inspired air to maintain SpO₂ ≥ 92%. Patients were intubated if they were deteriorating clinically and/or could not maintain SpO₂ ≥ 90% while breathing 100% O₂ via a face mask.

Statistical Analysis

Survival time was defined as the interval between onset of illness and death or censoring as of 9 June 2003. The following risk factors for mortality were studied: sex, age, race, co-morbidities, PaO₂/FiO₂ ratio on admission to ICU, lowest PaO₂/FiO₂ ratio, Acute Physiology and Chronic Health Evaluation (APACHE) II score, highest serum lactate dehydrogenase (LDH), lowest serum sodium, lowest serum potassium, lowest absolute lymphocyte count (ALC), highest total white count (TWC), highest prothrombin time (PT) and highest activated partial thromboplastin time (PTT).

It was observed that three-quarters of ICU deaths occurred on day 6 or later of ICU admission. Therefore, the relevant laboratory measurements within the first 5 days of admission to ICU were used in the analysis for prognostic factors. Univariate Cox regression models were fitted for each of the variables.

Starting from the most significant, variables that were significant in the univariate analysis were included in the multivariate model in

turn. The log-likelihood ratio test was used to test whether inclusion of a new covariate helped improve the fit of the model. The adjusted hazard ratios and their confidence intervals were provided for the final model.

The Nelson-Aalen cumulative hazard plot (on the log scale) was used to graphically examine the proportional hazard assumption. The Schoenfeld test was used to formally ascertain if the assumption of proportional hazards was violated for the final model. Data analysis was done in Stata (V6.0) and level of significance was set at 5%.

Results

The demographic and clinical characteristics of 46 critically ill probable SARS patients are shown in Table I. All the patients presented with fever; the other common symptoms were shortness of breath (89.1%) and cough (80.4%) (Table II). The list of co-morbidities is tabulated in Table III. The laboratory values within the first 5 days of ICU admission are shown in Table IV. Significant morbidity (Table V) and in-ICU of 52.2% (24/46) were observed.

The survival experience of the entire SARS ICU cohort is shown in Figure 1. Table VI highlights the univariate predictors for mortality. The hazard ratio was 2.6 (95% CI: 1.1 to 6.2) among male as compared to female ($p = 0.027$). The hazard decreased by 0.98 (95% CI: 0.97 to 0.99) for every unit increase in PaO₂/FiO₂ ratio ($p = 0.012$). Lowest PaO₂/FiO₂ ratio, APACHE II score, presence of congestive heart failure (CCF), cerebrovascular accident (CVA) and peptic ulcer were also found to be statistically significant.

In the multivariate analysis, only sex, APACHE II score and presence of CCF were found to be independently related to mortality (Table VII). Likelihood ratio tests indicated that none of the other predictors that were significant at the univariate analysis were significant, after adjustment for these covariates. The hazard ratio was 3.2 (95% CI: 1.2 to 8.4) among male as compared to female, after adjusting for APACHE II score and presence of CCF. The hazard

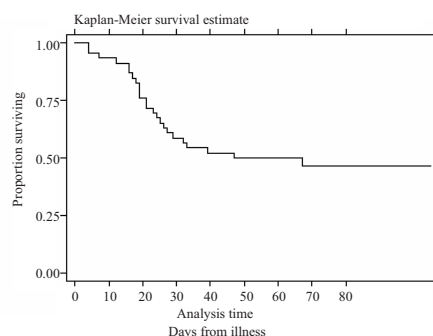


Fig. 1. Kaplan-Meier survival experience.

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TABLE I: DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF ICU PATIENTS (n = 46)

Variables	
No. of males (%)	24 (52.2)
Age (y)	
Mean (SD)	49.8 ± 16.4
Race [n (%)]	
Chinese	34 (73.9)
Malay	6 (13.0)
Indian	3 (6.5)
Others	3 (6.5)
No. intubated (%)	39 (84.8)
APACHE II	
Mean ± SD	19.2 ± 9.6
Length of ventilation (days)	
Median (IQR)	12 (9-22)
PaO ₂ /FiO ₂ ratio on ICU admission (mm Hg)	
Median (IQR)	88 (60-128)
Lowest PaO ₂ /FiO ₂ ratio (mm Hg)	
Median (IQR)	59.5 (44-95)
Days from symptom onset to hospitalisation	
Median (IQR)	5 (2-6)
Days from symptom onset to ICU admission	
Median (IQR)	8 (6-10)
Length of stay in hospital	
Median (IQR)	23.5 (15-36)
Length of stay in ICU	
Median (IQR)	14.5 (7-22)

TABLE II: SYMPTOMS EXPERIENCED BY ICU COHORT

Symptom	n	(%)
Fever	46	(100.0)
Shortness of breath	41	(89.1)
Cough	37	(80.4)
Myalgia	19	(41.3)
Diarrhoea	16	(34.8)
Malaise	15	(32.6)
Nausea/vomiting	14	(30.4)
Headache	12	(26.1)
Sore throat	9	(19.6)
Anorexia	6	(13.0)

TABLE III: LIST OF CO-MORBIDITIES

Co-morbidity	n	(%)
Asthma	2	(4.4)
Diabetes mellitus	7	(15.2)
Ischaemic heart disease	6	(13.0)
Hypertension	14	(30.4)
Congestive cardiac failure	3	(6.5)
Chronic renal failure	3	(6.5)
Cerebrovascular accident	1	(2.2)
Peptic ulcer	4	(8.7)
Others	22	(47.8)

ratios were 5.6 (95% CI: 1.7 to 18.1) and 9.5 (95% CI: 2.0 to 46.4) among those with APACHE II scores 15 to 30 and >30, as compared to those with a score of <15, after adjusting for sex and presence of CCF. The hazard ratio among patients with CCF was 21.7 (95% CI: 1.7-274.7) as compared to those without CCF, after adjusting for sex and APACHE II score. The Schoenfeld's test indicated that the assumption of proportional hazards was not violated for the overall model ($p = 0.627$). The assumption was also not violated for each of the covariate in the model.

Discussion

This coronavirus has no gender or racial predilection. Critically ill patients tend to be in their late middle age and required ICU admission in the second week of illness. All the patients required ICU admission because of respiratory failure, with median (inter-quartile range, [IQR]) PaO₂/FiO₂ of 88 (60 to 128) mm Hg. Eighty five percent of our ICU patients required intubation for mechanical

TABLE IV: LABORATORY VALUES WITHIN FIRST 5 DAYS OF ICU ADMISSION

Laboratory values	Mean ± SD
Highest serum LDH (U/L)	1429.4 ± 515.9
Lowest serum sodium (mmol/L)	131.20 ± 4.07
Lowest serum potassium (mmol/L)	3.4 ± 0.5
Lowest ALC (uL)	445.9 ± 186.0
Highest TWC (x 10 ⁹ /L)	12.1 ± 3.5
Highest PTT (s)	50.9 ± 18.4
Highest PT (s)	14.9 ± 2.2

TABLE V: LIST OF COMPLICATIONS

Complications	n (%)	95% CI
Septicaemia	12 (26.1)	(14.3-41.1)
Secondary pneumonia	24 (52.2)	(36.9-67.1)
Pneumothorax	8 (17.4)	(7.8-31.4)
Pulmonary embolism	7 (15.2)	(6.3-28.9)
Deep vein thrombosis	11 (23.9)	(12.6-38.8)
Cerebrovascular accident	4 (8.7)	(2.4-20.8)
Acute renal failure	9 (19.6)	(9.4-33.9)
Pneumomediastinum	1 (2.2)	(0.06-11.5)

95% exact confidence interval from binomial distribution

TABLE VI: UNIVARIATE PREDICTORS OF MORTALITY

Covariates	Hazard ratio	95% CI	p value
Sex (Male)	2.63	(1.12, 6.18)	0.027
Age (y)	1.02	(0.99, 1.05)	0.117
Race			
Chinese	1		
Malay	0.83	(0.24, 2.82)	0.762
Indian	1.02	(0.24, 4.39)	0.981
Others	0.47	(0.06, 3.52)	0.462
PaO ₂ /FiO ₂ ratio on admission to ICU	0.98	(0.97, 0.99)	0.012
Lowest PaO ₂ /FiO ₂ ratio	0.99	(0.97, 0.99)	0.040
APACHE II score			
<15	1		
15-30	3.83	(1.25, 11.72)	0.019
>30	18.18	(4.66, 71.00)	<0.001
Highest serum LDH			
Up to 1000 (U/L)	1		
>1000 (U/L)	3.33	(0.98, 11.29)	0.053
Lowest serum sodium			
>136 (mmol/L)	1		
Up to 136 (mmol/L)	0.80	(0.19, 3.41)	0.764
Lowest serum potassium			
>3.5 (mmol/L)	1		
Up to 3.5 (mmol/L)	0.73	(0.33, 1.63)	0.444
Lowest ALC (ul)	1.00	(0.99, 1.00)	0.625
Highest TWC (x10 ⁹ /L)			
Up to 10	1		
>10	2.25	(0.93, 5.45)	0.072
Highest PTT (s)			
Up to 43	1		
>43	1.57	(0.62, 3.96)	0.339
Highest PT (s)			
Up to 14.5	1		
>14.5	1.83	(0.82, 4.09)	0.14
Ischaemic heart disease	2.40	(0.89, 6.46)	0.083
Hypertension	1.44	(0.63, 3.30)	0.385
Congestive cardiac failure	79.75	(7.81, 813.9)	<0.001
Diabetes mellitus	1.61	(0.60, 4.32)	0.346
Cerebrovascular accident	14.57	(1.52, 140.13)	0.020
Peptic ulcer	5.45	(1.82, 16.26)	0.002

TABLE VII: MULTIVARIATE PREDICTORS FOR MORTALITY

Covariates	Hazard ratio	95% CI	p value
Sex (Male)	3.18	(1.20, 8.40)	0.019
APACHE II score			
<15	1		
15-30	5.60	(1.73, 18.07)	0.004
>30	9.52	(1.95, 46.44)	0.005
Congestive cardiac failure	21.70	(1.71, 274.74)	0.017

ventilation.

Reports from Hong Kong,¹¹⁻¹³ Canada^{6,9} and Singapore¹⁴ showed that 19% to 32% of SARS patients required ICU admission. About 13.8% to 25.3% of patients were mechanically ventilated.^{6,9,11,13,15} Reported mortality ranges from 3.6% (at day 21 of outbreak)¹² to 10.1% (at day 28 after symptom onset).¹⁴

In-ICU mortality ranges from 37% (at day 28 after symptom onset)¹⁴ to 34.2% (at day 28 after ICU admission).⁹ In the ICUs in Toronto, mortality at 8 weeks was 52% for those requiring mechanical ventilation,⁹ which is consistent with the mortality rate of ARDS patients requiring mechanical ventilation.¹⁶ In our study, 61.5% (24/39) of ventilated patients died.

The overall mortality for SARS patients was 13.2% for those below 60 years and 43.3% for those aged 60 or older.¹¹ Data from Hong Kong indicated that advanced age, peak LDH and an initial elevated white cell count may carry a poor prognosis.¹² Unpublished data from our local experience estimated that mortality for patients below 60 years was 8.4%, and for those aged 60 or older was 50%. Age and serum LDH values on admission were predictors for ICU admission for our local SARS patients.

A paper from Toronto showed that diabetes and other co-morbid conditions were independently associated with poor outcome, but age of 60 years older was not.⁶ Our study indicated that risk factors for ICU mortality were male gender, APACHE II score ≥ 15 and history of congestive cardiac failure.

Although mortality is higher in older patients particularly those with pre-existing co-morbid illness, previously young healthy persons have also succumbed to the disease. Some authors postulated this to higher viral loads or their host hyperimmune response.¹⁷ To control the severe inflammatory damage, corticosteroids alone^{18,19} or in combination with immunoglobulin have been attempted, including our centre. Such therapy, if proven to be effective, may be targeted at patients with poor prognostic risk factors.

Comparison of mortality among different cohorts is difficult because of different completeness of follow-up and case definition of SARS.⁶ Only 6 (3%) out of 199 in our TTSH cohort remained hospitalised at the end of the study period, compared to 45% and 23% in Hong Kong¹² and Canada⁶ respectively. The case fatality rate is approximately 3% to 12%, depending on whether the denominator includes both suspect and probable cases (3%) or probable cases alone (12%).^{7,8,12,17}

The WHO case definition for probable SARS was revised on 1 May 2003 to include polymerase chain reaction to detect the virus and/or serology to detect the antibodies as criteria for defining a probable SARS case.²⁰ Singapore was declared SARS-free by WHO on 31 May 2003. The last SARS patient was discharged from hospital on 13 July 2003. The final overall case fatality in Singapore is 13.7% (33/238), following reclassification of 32 out of 600 suspect cases as probable on the basis of diagnostic laboratory tests.^{21,22} The most recent WHO epidemiological record dated 27 June 2003 reported an overall case fatality of 15%.²³

Conclusion

Critically ill SARS patients had significant morbidity and mortality. They also consumed much ICU resources. Such information are useful for the clinicians and health administrators in planning for future outbreaks of SARS. Further studies are needed to elucidate the poor prognostic factors so that novel therapy can be targeted in such high-risk patients, to improve the outcomes and reduce the burden of the disease on the health care service.

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Both Gram Positive and Gram Negative Organisms Complicate Neutropaenic Sepsis in Children with Leukemia. A Unit Audit Study and Recommendation

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Introduction

Neutropaenic sepsis is the most important cause of preventable mortality in children with cancer.¹ Early institution of empiric broad spectrum antibiotics is the cornerstone of childhood cancer therapy for the last 30 years and has reduced mortality attributed to sepsis from 30% to 1% currently.²

As we pursue increasing cure rates with more intensive multi-agent chemotherapy and the widespread use of long-term central venous catheters, breaches in the skin and mucosa together with prolonged severe neutropaenia actually increases the risks of neutropaenic sepsis. Fortunately, mortality from neutropaenic sepsis continues to decrease to very low levels of 1%. This is possible because of the constant review of the *current* bacteriological pattern in the treatment centre so that the appropriate empiric broad spectrum antibiotics can be instituted to adequately cover these organisms until blood culture and sensitivities come back.

Our choice of empiric antibiotics have changed over the years from ceftazidime/amikacin to the current ceftriaxone monotherapy. We conducted a retrospective audit study of the patients admitted with neutropaenic fever and their culture/sensitivity results to assess whether our current ceftriaxone monotherapy is sufficient.

Materials and Methods

This is a retrospective study of children with leukaemia admitted for neutropaenic fever over a 2-year period from January 2001 to January 2003. We collected data on demographic characteristics, disease status and the number of admissions for febrile neutropaenic episodes among children with leukaemia. A review of all cultures taken for the patients was done and microbiological laboratory results were collected to determine the pattern of organisms isolated.

Results

Twenty-eight children with leukaemia (Table I) were admitted for neutropaenic fever during the study period. The median number of

TABLE I: PATIENT CHARACTERISTICS

Characteristics	No. of patients
Total no. of patients	28
ALL	27
CML	1
Age (y)	
Median	5
Range	1½-14
Sex ratio	
Male: female	1.2:1
Disease status	
Remission	14
Relapse	14
No. of admissions for neutropaenic fever	
Median	4
Range	1-15

TABLE II: TYPE OF CULTURES TAKEN DURING FEBRILE NEUTROPAENIC EPISODES

Type of culture	No. of cultures	No. of positive cultures
Blood	213	32 (15%)
Wound	37	10 (27%)
Respiratory	14	2 (14%)
Urine	44	7 (16%)
Stool	30	3 (10%)

admissions for neutropaenic fever was 4 (range 1-15). There was an equal number of patients in remission and relapse (n = 14 each) with a slight male predominance (M:F 1.2:1.0). The median age was 5 years (range 1½-14 years). Majority of cultures taken during admission were from blood (n = 213) and other cultures were from urine (n = 44), wound/exit site (n = 37), stool (n = 30) and respiratory (n = 14).

Table II shows the overall positive cultures from the various sites. Fifteen percent of blood cultures yielded positive results. The highest positive yield came from cultures obtained from wound and central venous line exit sites (27%). Respiratory and stool cultures had only 14% and 10% culture positive results respectively.

Interestingly, there was a predominance of gram positive cocci infections (54%) compared with gram negative bacteria (46%) isolated in blood cultures. *Streptococci* spp was the single most common organism cultured in our patients, followed by *Staphylococcus aureus* and *Pseudomonas*. As expected, urine grew mostly gram negative microorganisms. Fungal microorganisms were uncommon but were the cause of death in 1 patient. Another patient died of multi-resistant *Pseudomonas aeruginosa* and progressive disease.

Discussion

Infection in the neutropaenic patient has remained a major preventable cause of mortality in cancer patients.¹ Institution of early empiric antibiotics during neutropaenic fever is one of the most important reasons for improvement in cure rates of childhood leukaemia over the last 30 years. Though there is a daunting list of possible types of organisms that can cause sepsis in an immunocompromised host, there is a recurrent pattern of organisms seen during the first days of presentation of neutropaenic fever.³ This reflects the pathogenic nature of such organisms and the breach in the skin and mucosa as the portal of entry for infections.

A constant audit of the pathogens cultured in each cancer unit is crucial in order to ensure that appropriate empiric antibiotics are used. With the widespread use of central lines and more intensive regimens that causes severe mucositis, there has been a shift from the previous predominance of gram negative sepsis to predominantly gram positive bacteraemia in this study.

Lee et al⁴ from our department studied 113 children with leukaemia from 1974-1984 and found that gram negative organisms accounted

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TABLE III: MICROORGANISM PATTERN AMONG POSITIVE CULTURES.

Type of cultures and microorganisms	No. of positive cultures
Blood cultures	26
Gram positive	14
<i>Streptococcus</i>	9
<i>Staphylococcus aureus</i>	4
<i>Gemella morbillorum</i>	1
Gram negative	12
<i>Pseudomonas</i>	4
<i>E. coli</i>	3
<i>Campylobacter</i>	1
<i>Enterobacter</i>	1
<i>Stenotrophomonas</i>	1
<i>Klebsiella/Pseudomonas</i> (mixed)	1
<i>Streptococcus/Klebsiella</i> (mixed)	1
Wound/exit site cultures	10
<i>Staphylococcus</i>	4
<i>Klebsiella</i>	2
<i>Pseudomonas</i>	2
<i>E.coli</i>	1
<i>Bacteroides</i>	1
Respiratory cultures	
<i>Candida/Aspergillus</i>	1
Urine cultures	6
<i>Pseudomonas</i>	2
<i>Proteus</i>	1
<i>E.coli</i>	1
<i>Klebsiella</i>	1
<i>Klebsiella/Pseudomonas</i>	1
Stool cultures	2
<i>Campylobacter</i>	1
<i>Clostridium</i>	1

for 53% (40/75) of episodes of bacteraemia. In this study in the same cohort of predominantly acute lymphoblastic leukaemia (ALL) patients, we found that gram negative organisms now account for only 46% of all bacteraemic episodes. There is a distinct shift in organism profile from gram negative organism to coagulase negative staphylococcus⁵ in 1992-1996 and now to streptococci in 2001-2003.

In addition, *Pseudomonas* sp has emerged to be the most common gram negative organism isolated (33% of gram negative organisms) in this study compared to the 1992-1996 cohort (18% of gram negative organisms isolated).⁵ This prompted us to change our empiric antibiotic of ceftriaxone monotherapy to ceftazidime/amikacin combination. Interestingly, *Streptococci* have now emerged to be the most important common pathogen (35% of all bacterial isolates and 64% of all gram positive isolates). Fortunately they are uniformly sensitive to penicillin, ceftriaxone and ceftazidime. The increasing importance of *Streptococci* may be from the presence of severe mucositis as a result of therapy. Fortunately we have not seen an increasing incidence of ARDS⁶ that results from these *Streptococci*.

Coagulase negative *Staphylococci* have decreased in importance as improved central line care has been instituted.⁷

Though it is intuitive that we institute the broadest spectrum and most powerful antibiotics in our armamentarium empirically so that most bacteria will be covered, this drastically increases treatment costs and worse still encourage development of resistant organisms. For example, our preliminary foray into using meropenem first line for some neutropaenic patients resulted in selection of multi-resistant *Sternotrophomonas maltophilia* in some patients. Moreover in the current era of disease-related group (DRG) coding and reimbursements, proper use of antibiotics through carefully audited culture results is crucial.

Conclusion

Blood cultures comprise a majority of cultures taken during febrile neutropaenic episodes. Among positive blood cultures, both gram positive and gram negative organisms are emerging as equally important pathogens and this should be considered in deciding for antimicrobial coverage. The increasing use of more intensive chemotherapy with its accompanying severe mucositis may increase the incidence of streptococci infections and should also be taken into consideration. Future studies should focus on increasing the yield in blood cultures to aid in the management of children with neutropaenic fever.

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Ambulatory Care of Central Venous Hickman's Catheters Can Be Successfully and Safely Carried out by Parents at Home: Results of a Patient Education Programme

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Introduction

Pain and vomiting are the usual images that haunt a child and family when they are told that the child is stricken with cancer. Pain, arising from the need to set IV plugs for chemotherapy and regular blood-taking for blood counts and chemistries, is probably one of the most stressful problems for children with cancer and their families. Though central venous lines ensure more reliable and safer administration of chemotherapeutic drugs by minimising extravasation that can cause severe skin necrosis, for children with cancer they are both a big blessing and their best friends. Central venous lines remove the need for painful needle sticks and free the child's hands to play, write and draw.

Hickman's catheters (HCs) are double lumen, cuffed, tunneled central venous catheters frequently used in the care for children with cancer. Though HCs are welcome adjuncts to cancer care, they can create considerable morbidity and mortality if not used and cared for properly.^{1,2} Because HCs are externalised, they provide an easy route for bacteria to gain direct access into the bloodstream, causing septicaemia, infective endocarditis and even death. In addition, because it is a foreign body in the superior vena cava, it is prone to cause both thrombosis and embolism with dire consequences.

These are the reasons why HCs are managed with great caution by nurses. They require frequent aseptic cleansing, exit site skin care and flushing with heparinised saline at least twice a week. This entails added costs, inconvenience and repeated trips to the hospital by both the patient and parents/caregiver, as well as increased nursing burden for nurses.

In 1997, as part of our Home Care Programme and our aim to empower the patients and their parents to participate actively in their cancer care, we reviewed the standards of care for children with central venous lines and initiated the teaching of the basic principles of Hickman's line care to parents. This initially started as an unstructured discussion by nurses with parents and transferred the responsibility of home care for HCs to parents/caregivers. A year into the programme, we reviewed and showed that patients suffered a high rate of 11 infections per 1000 catheter days and required removal of many catheters.³ This led to a review of our Home Care Programme and the development of a systematic patient education programme for the training of caregivers, particularly parents, in the management of central venous line catheters at home. We describe our patient education programme for the home care of HCs in children with cancer and compared the incidence of infection sepsis in children with HCs before and after implementation of this programme.

Materials and Methods

Parents underwent a "Procedures Made Easy for Parents" Patient Education Programme conducted by nurses that emphasised simple

techniques on flushing HC and exit site dressings. A teaching plan was prepared with all the important aspects of each session clearly written out. Teaching aids were designed to ensure that a life-like demonstration can be carried out and a checklist was designed to track and monitor performance of nurses and parents/caregivers. Parents are guided until assessed to be capable and confident to conduct the procedures in front of the training nurse before they are allowed to administer care independently at home.

Subsequently, parents/caregivers performed skin care and flushing of HC by themselves at home, with regular home follow-up by the nurses who reviewed their techniques. Parents can call up for queries and come directly to the Paediatric Inpatient Cancer Centre if problems arise. A complete central patient database with central venous catheters was maintained and the complications tracked.

A retrospective study was done from January 1998 to October 2002 to determine the outcome of home care for HCs after the start of an intensive training programme. Data were collected encompassing demographic characteristics, underlying malignancy, neutrophil count on day of device insertion and presence of neutropaenia (absolute neutrophil count <1,000 cells/uL). A review of microbiology laboratory records was conducted to determine infectious complications. This included blood culture/exit site/catheter tip results and haematological parameters during admission. The outcome of therapy and reason for catheter removal were ascertained. The National Nosocomial Infection Surveillance System criteria for primary bloodstream infections were used to define bacteraemic episodes and bloodstream infection (BSI) rates were calculated by the number of BSI multiplied by 1,000 and divided by the number of HC days.⁴

Results

Forty children with cancer had a HC inserted from January 1998 to October 2002 during the implementation of the "Procedures Made Easy for Parents" Education Programme at our unit. Patients had a median age of 13.5 years (range 1-18) with male-to-female ratio of 3:2. 73% (n = 29) of these children had leukaemia and 27% (n = 11) had solid tumours; 33% (n = 13) of patients underwent stem cell transplantation. Parents cared for 30 (75%) patients with HC. The median duration of HC remaining *in situ* was 132.5 days (range 19-710) (Table I). The infection rate for HC was 2.41 per 1000 catheter days with relative risk for bloodstream infection at 2.13. Majority of infections were with gram negative bacteria, accounting for 1.7 bloodstream infections per 1,000 catheter days (Table II). Reasons for removal of HC include infection [10% (n = 4)], mechanical causes [5% (n = 2)], completion of therapy [48% (n = 19)] and mortality [33% (n = 13)]. Only 1 mortality was related primarily to sepsis. Two catheters were still in place at time of censor of follow-up.

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Discussion

Painful needle sticks are one of the most stressful problems confronting children with cancers. Central venous lines like HCs are welcome adjuncts to therapy minimising painful needle sticks and freeing the child's hands for play. However, HCs have a higher potential for infection due to its externalised position. Thus, HCs require frequent care which includes flushing, exit site care and changing the dressing twice a week.

Though, with proper Hickman's line care, the rate of infections can be greatly minimised to acceptable levels, this will require frequent trips to the hospital, which may be troublesome to parents and incur added cost to patients. Traditionally, nurses are singularly involved in the care of HCs. As more catheters are placed, increased burden is placed on the already heavy nursing loads.

In 1997, as part of our Home Care Programme and Total Cancer Care where parents are empowered to participate actively in their child's cancer care, we embarked on a parent-administered HC care at home. This was fraught with higher infection rates for HCs (11 per 1000 catheter days) compared to Porta-caths (3.1 per 1000 catheter days).³ Porta-caths do not require any home care compared to HCs.

The programme was redesigned to incorporate an intensive Parents Education Programme that involved comprehensive information for parents, teaching plan and aids, a performance checklist and close follow-up of parents/caregivers. Teaching aids that facilitated life-like demonstration, such as actual withdrawal of blood-like fluid

(red-coloured food dye mixed with water), were effective tools for learning.

We also procured a mannequin named Chester who has multiple detachable layers to illustrate how the HC works and how infections can be acquired. A practical demonstration is conducted that allows parents/caregivers to learn the techniques step by step and a checklist ensures that all areas of learning are covered. This is to guide the teacher throughout the session, thus increasing inter-teacher reliability. Once the parent/caregiver is deemed confident and competent, they are allowed to take care of the HC at home by themselves. Nurses conduct frequent home follow-ups to ensure adherence to technique and to deal with problems that may arise. In addition, parents/caregivers have direct 24-hours' access to the wards for questions via the telephone or can come directly to the Paediatric Inpatient Cancer Centre if any problems arise.

After implementation of this systematic "Procedures Made Easy for Parents" Patient Education Programme, we found that 75% (30/40) of catheters could be cared for by the parents/caregivers at home. The incidence of HC-related infections has been reduced from the historical 11 per 1000 catheter days³ to 2.41 per 1000 catheter days which is marginally higher than that for Porta-caths (0.85 per 1000 catheter days) during the same study period.

In our previous³ and current studies, we found that HCs have higher infection rates than Porta-caths. This is attributed to the fact that Hickman's lines are externalised and have 2 lumens, rather than a single lumen. If we adjust for the differences in lumen numbers, HCs have 1.2 infections per lumen per 1000 catheter days, which is minimally higher than Porta-caths (0.85) (Table III).

Conclusion

Majority of parents/caregivers can manage HCs with minimal risks of infection after an intensive educational programme. Our programme significantly reduces the inconvenience of repeated trips to the hospital without compromising catheter safety.

Acknowledgement

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TABLE I: SUMMARY OF PATIENT CHARACTERISTICS AND OUTCOME OF HCs

Characteristic	
Total no. of patients	40
No. of HC taken care by parents	30
Age (y)	
Median (range)	13.5 (1-18)
Sex (M:F)	3:2
Underlying diagnosis	
Haematological	29
Solid tumour	11
Bone marrow transplant	13
Neutrophil counts on day of insertion	
Median	2.46
Range	0.89-24.47
Neutropaenia	24
Site infection	0
HC removal	
2° to infection	4
2° mechanical causes	2
2° completion of therapy	19
2° mortality	13
Mortalities related to sepsis	1

TABLE II: BLOODSTREAM INFECTIONS CAUSED BY HC

Characteristic	
Duration of use (days)	
Total no. of days	7041
Median	132.5
Range	19-710
No. of BSIs	17
per 100 catheters	42.5
per 1,000 catheter days	2.41
No. of gram-negative BSIs	12
per 100 catheters	30
per 1,000 catheter days	1.70

BSI: bloodstream infection

TABLE III: COMPARISON OF CURRENT STUDY RESULTS WITH PRIOR PATIENT EDUCATION PROGRAMME

Study	Current		Huang et al ³	
	Hickman	Ports	Hickman	Ports
No of bloodstream infections				
Per 100 catheters	42.5	20	ND	ND
Per 1000 catheter days	2.41	0.85	11.0	3.1
Per lumen per 1000 catheter days	1.20	0.85	5.5	3.1

Pain Management at the Emergency Department—Can We Do Better?

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Introduction

The Emergency Department (ED) at Tan Tock Seng Hospital (TTSH) sees about 350 patients a day. It also received the highest number of ambulance cases amongst all the hospitals in Singapore. 15% of these cases present with a chief complaint of pain.

Our sequential approach to patient flow does not allow early intervention to deliver analgesia at the earliest possible time. A 2-week review of all cases presenting with pain to our ED showed that 55% of these patients received no analgesia. It was also found that the mean waiting time from registration to the time of administration of analgesia was 71.4 ± 45.7 minutes.

The aims of this study were:-

1. To study the perception of pain amongst patients by gender, age, race and type of injury;
2. To determine how the above factors affected decision by patient with regards to analgesia at triage; and
3. To determine the level of pain on the pain rating scale whereby patients would prefer analgesia to be given at the point of triage.

Materials and Methods

The study was conducted from 26 November to 7 December 2002. All patients presenting with pain secondary to minor injuries like abrasions, lacerations and contusions, and more severe injuries, like fractures and/or dislocations, were prospectively recruited. Patients were interviewed via a structured questionnaire.

Patients were asked to indicate on the Numeric Rating Scale (NRS) the pain they felt. They were then asked whether they wanted analgesia while they were waiting to be seen by the doctor and how long they thought was a reasonable time to wait before analgesia was given.

The patient's education level, occupation and financial background were also recorded. All doctors were blinded to the pain score as well as the answers to the study questionnaire. Records were later reviewed to see if patients received analgesia at the ED and how long it took before they received it. The results were analysed using SPSS for Windows 11.0.

Results

Of the 175 eligible patients, 167 patients agreed to participate while 8 declined. Of these 167 patients, 157 of them indicated their decision for analgesia at triage while 10 did not (69 wanted, 88 declined and 9 were unsure and 1 patient did not respond to the question). 54 (32.3%) patients were given analgesia while at the ED while 113 (67.7%) patients were not.

There were 130 (77.8%) male and 37 (22.2%) female patients. The median pain score was 6 (range 0-10) for both genders. No statistical significance in pain score ($p = 0.313$, Mann Whitney *U* test).

The mean age was 33.3 years (95% CI: 35.5 to 35.1). No significant correlation was found between the age of the patients and their pain score ($r = 0.075$, Spearman's correlation co-efficient).

There were 88 Chinese (52.6%), 14 Malays (8.4%), 37 Indians (22.1%) and 28 other races (16.7%). The Chinese, Malay, Indian and other races had a median pain score of 6 (range 0-10), 5 (range 3-10),

8 (range 1-10) and 6.5 (range 3-10) respectively. Indian patients had a significant higher median pain score when compared to the all other races ($p = 0.036$, Kruskal-Wallis test; adjusted)

No significant correlation was found between the NRS score of patients with different educational levels ($r = 0.004$, Spearman's correlation co-efficient) and financial background ($r = 0.053$, Spearman's correlation co-efficient)

There were 118 patients (70.7%) who suffered minor injuries and 44 patients (26.3%) who sustained fractures and/or dislocations. The median pain score was 6 (range 0-10) for those with minor injuries and also 6 (range 1-10) for those with fractures and/or dislocations. This was not statistically significant ($p = 0.99$, Mann Whitney *U* test). 5 (3%) of the cases had no indication of the nature of injury.

69 (41.3%) out of the 167 patients wanted analgesia at triage. Patients wanting analgesia had a median pain score of 7 (range 0-10) while those who declined had a median pain score of 5 (range 0-10). This difference was statistically significant ($p = 0.008$, Mann Whitney *U* test; adjusted).

The median pain score for patient's who received analgesia was 7 (range 3-10) while those who did not was 6 (range 0-10). This was statistically significant ($p = 0.01$, Mann Whitney *U* test; adjusted).

Amongst 69 patients wanting analgesia while waiting to be seen, 34 (49.3%) patients received analgesia while the remaining 35 (50.7%) did not. Of the 88 patients who declined analgesia while waiting to be seen, 20 (22.7%) patients received analgesia after consultation while 68 (77.3%) received no analgesia at all. This difference was statistically significant ($p = 0.022$, chi-square test; adjusted).

More Indian and "other races" patients indicated that they wanted analgesia at triage (Proportion wanting analgesia: Chinese = 30.2%, Malay = 50%, Indian = 63.6%, other = 62.5%; $p = 0.002$, chi-square test; adjusted) but this did not alter the proportions that subsequently received analgesia (Chinese = 30.7%, Malay = 28.6%, Malay = 28.6%, Indian = 37.8%, other = 32.1%; $p = 0.65$, chi-square test).

The gender, age and type of injury did not affect the patient's decision for analgesia (gender; $p = 0.45$, chi-square test, age; $p = 0.65$, unpaired t-test, type of injury; $p = 0.58$, chi-square test) or subsequent receipt of analgesia (gender; $p = 0.86$, chi-square test, age; $p = 0.69$, unpaired t-test, type of injury; $p = 0.35$, chi-square test).

The mean waiting time from arrival at the ED to the time patient received analgesia was 77.6 ± 43.2 minutes. Patients' expectations of waiting time for the delivery of analgesia did not change with their decision on whether they wanted analgesia at triage or not. 105 (66.7%) expected analgesia to be given within 15 minutes of their arrival at the ED.

Discussion

In our study, race had a significant effect on the median pain score. The proportion of Indian patients and patients of "other races" who wanted analgesia were also significantly higher. It was a common observation amongst our healthcare workers that patients from the Southern Asia region tend to have a lower threshold for pain

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compared to the other races. They are also more demonstrative of their painful experience and more inclined to want analgesia.

In our study, 69 (41.3%) patients indicated that they wanted analgesia while waiting to be seen by the doctor. However, only 34 (49.3%) received analgesia while the other 35 (50.7%) did not. The low analgesia prescription rate amongst those who indicated that they wanted analgesia is a cause of concern. We propagated that under treatment of pain could be due to no formal education in pain management and our current work processes. Hence, we need to inculcate in our staff the importance of pain management, and to understand the physiologic aspects of pain from the patients' perspective.

Our current work processes did not allow us to meet the patients' expectation of delivery of analgesia at the ED. The patients expected analgesia to be given within 15 minutes of their arrival at the ED. In a busy ED like ours, patients presenting with pain secondary to minor injuries, fractures and/or dislocations usually have a longer waiting time to be seen by a doctor as these injuries are not life threatening. While it is important for doctors and nurses to attend to life threatening conditions, it is also our duty to relief patient's pain at the earliest possible time.

The implementation of a protocol-based nurse delivery of analgesia and process evaluation are possible approaches to meet patient's expectation for the timely delivery of analgesia while not

compromising our care for patients with emergent life threatening conditions.

It is not possible to compare pain intensity between 2 individuals. One may assume that the intensity of trauma have an effect on the pain score. However, our study showed that intensity of trauma had no significant difference on the patient's pain score, preference for analgesia or subsequent receipt of analgesia. Pain is a subjective expression and can be affected by many factors like gender, age, race and psychosocial background. The pain score will act as a guide in stratifying patients in need of analgesia at triage.

Conclusion and Recommendation

We need to educate our healthcare workers on the importance of assessment and treatment of pain. The time to delivery of analgesia fell short of our patients' expectations. Our current workflow does not allow us to meet the patients' need and expectation for the timely delivery of analgesia. Therefore, it is important to improve our processes in the management of pain and to develop a protocol driven parenteral analgesia delivery mechanism whereby nurses are permitted to prescribe analgesia at triage. Assessing pain using the NRS is useful and this should be incorporated as the fifth vital sign in the assessment of all patients at triage. Patients with a pain score of 7 should be offered analgesia at triage and those with a pain score of 5 or less should only be given analgesia at triage if they requested for it.

Identification of Factors Influencing Caregiving Experience in Caregivers of Patients with Schizophrenia

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Introduction

Caregivers of a family member with schizophrenia are undoubtedly affected by their caregiving roles.¹⁻⁴ In a previous study, we found that many such caregivers in Singapore experienced clinically important distress.⁵ However, factors that influenced caregiving experience of these caregivers are unknown. The purpose of this study was to identify factors that influence caregiving experience in caregivers of a patient with schizophrenia in Singapore.

Materials and Methods

Study Design

A consecutive sample of outpatients with schizophrenia and their caregivers seen in the Institute of Mental Health were recruited for this IRB-approved study. After written consent was obtained, caregivers and patients were surveyed individually during routine counseling by 2 medical social workers (MSWs) who were proficient in both English and Chinese. Caregivers were interviewed using a questionnaire containing the Experience of Caregiving Inventory (ECI)⁶ and questions assessing caregiving activities, socio-demographic status and support from various sources in the preceding 3 months. The patients completed a questionnaire containing the Schizophrenia Quality of Life Scale (SQLS),⁷ the Short Form 36 Health Survey (SF-36)⁸ and questions assessing socio-demographic status. The patients were also assessed for presence of 22 psychiatric symptoms and their utilisation of medications and inpatient services in the preceding 3 months was examined using medical records. All questionnaires were prepared in identical English and Chinese versions for use at interviewees' preference.

Inclusion criteria for patients were: 1) outpatients with a previous diagnosis of schizophrenia; 2) well enough to cooperate with the study; and 3) ability to communicate in English or Chinese. Inclusion criteria for caregivers were: 1) caregiver of a patient with schizophrenia; and 2) ability to communicate in English or Chinese.

Instruments

The ECI measures the experience of caring a person with a serious mental illness such as schizophrenia. The ECI consists of 66 items which provide a comprehensive description of caregiving experience in 8 negative dimensions (i.e. difficult behaviours, negative symptoms, stigma, problem with services, effects on the family, need to back up, dependency and loss) and 2 positive dimensions (i.e., rewarding personal experiences and good aspects of relationship with the patient). These dimensions can be consolidated into a negative total score (score range: 0 to 208, higher scores indicating more negative experience) and a positive total score (score range: 0 to 56, higher scores indicating more positive experience).⁶ The recall period of the ECI is the past 1 month. Both English and Chinese versions of the ECI have been validated in Singapore.⁹

The SQLS is a 30-item self-report questionnaire which needs 5 to 10 minutes to complete.⁷ The instrument measures schizophrenia-specific quality of life/ health status in 3 dimensions (Table I). The

recall period of the instrument is the past 7 days. Both English and Chinese versions of the SQLS have demonstrated acceptable psychometric properties in Singaporeans with schizophrenia.¹⁰

The SF-36 is a 36-item self-report questionnaire which can be administered in 5 to 10 minutes.⁸ The instrument measures self-perceived health status and functioning in 8 dimensions (Table I). The recall period of the SF-36 used in this study was the past 4 weeks. The SF-36 has been intensively validated in many countries including Singapore.¹¹⁻¹³ Both English and Chinese versions of the SF-36 were validated in Singaporeans with schizophrenia.¹⁰

The psychiatric symptom checklist is designed for trained MSWs to assess patients with schizophrenia for presence or absence of 22 psychiatric symptoms. These symptoms are misery/low spirit, social withdrawal, slowness in movement/activities, forgetfulness, underactivity, over-dependence, unable to make decisions, worrying frequently, unusual fear, compulsive behaviours, delusions/hallucinations, hyperactivity, unpredictability, irritability, rudeness/inconsideration, verbal/physical violence, suicidal expressions/behaviours, offensive/inappropriate sexual behaviours, heavy drinking, lack of self-care, bodily aches/pains and odd behaviours.

Statistical Analysis

Multiple linear regression analysis with stepwise methods was used to identify factors that predict caregiving experience measured by the ECI. Thirty-nine variables were studied using 2 multiple linear models, with the ECI negative total scores and the ECI positive total scores being dependent variables respectively. Independent variables covered socio-demographic, health-related and clinical characteristics of the caregivers and/or the patients (Table I). Among these variables, age, duration of caregiving, age of onset of schizophrenia, duration of schizophrenia, number of psychiatric symptoms, expense on psychiatric therapies and patients' self-report health status (measured by the SQLS and SF-36) were treated as continuous variables while the remaining as categorical variables. The criterion significance levels for entry and removal of an independent variable for each step of modelling were chosen to be 0.05 and 0.10, respectively. Analyses were performed with SPSS for Windows (version 11.5).

Results

Subject Characteristics

Caregivers: One hundred and fifty-nine eligible caregivers participated in the study. The mean (SD) age of the caregivers was 52.4 (14.1) years, with 58.5% being female. Fifty-two percent of the caregivers were parents of patients and the mean (SD) duration of caregiving was 7.2 (6.9) years. Full socio-demographic characteristics of the caregivers are shown in Table II.

Fifty-six percent, 91.8% and 66.0% of the caregivers reported that they received emotional or practical support from family/friends, spiritual/traditional healers and medical professionals, respectively, in the preceding 3 months. The mean (SD) ECI negative total and

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TABLE I: LIST OF INDEPENDENT VARIABLES

Variable	Category
Socio-demographic variable	
Age of caregiver	Continuous
Age of patient	Continuous
Gender of caregiver	Male, female
Gender of patient	Male, female
Ethnicity of caregiver	Chinese, others
Marital status of caregiver	Married, others
Marital status of patient	Married, others
Caregiver-patient relationship	Parent, others
Duration of caregiving	Continuous
Employment status of caregiver	Employed, unemployed, homemaker
Employment status of patient	Employed, unemployed, homemaker
Education level of caregiver	Primary, secondary, tertiary
Education level of patient	Primary, secondary, tertiary
Survey language of caregiver	English, Chinese
Survey language of patient	English, Chinese
Support from family and friends*	Yes, no
Support from spiritual/traditional healers*	Yes, no
Support from medical professionals*	Yes, no
Health-related variable	
Caregiver's chronic medical conditions	Yes, no
Patient's chronic medical conditions	Yes, no
Psychosocial (SQLS scale)	Continuous
Energy/ motivation (SQLS scale)	Continuous
Symptoms/ side effects (SQLS scale)	Continuous
Physical functioning (SF-36 scale)	Continuous
Role limitation due to physical problems (SF-36 scale)	Continuous
Bodily pain (SF-36 scale)	Continuous
General health (SF-36 scale)	Continuous
Vitality (SF-36 scale)	Continuous
Social functioning (SF-36 scale)	Continuous
Role limitation due to emotional problems (SF-36 scale)	Continuous
Mental health (SF-36 scale)	Continuous
Patient's clinical variable	
Age of onset of schizophrenia	Continuous
Duration of schizophrenia	Continuous
Number of psychiatric symptoms	Continuous
Recent admission in a psychiatric ward*	Yes, no
Usage of typical antipsychotics*	Yes, no
Usage of atypical antipsychotics*	Yes, no
Usage of depot antipsychotics *	Yes, no
Expense on psychiatric therapies*	Continuous

* In the preceding 3 months.

positive total scores were 77.6 (31.8) and 29.2 (9.8) points, respectively.

Patients: The mean (SD) age of the patients (n = 159) was 37.2 (10.8) years, with 50.3% being female and 58.5% surveyed on the same day when their caregivers were surveyed. The mean (SD) age of onset of schizophrenia was 29.2 (10.9) years. The majority of the patients were unmarried (69.2%) and unemployed (64.2%). Full socio-demographic characteristics of the patients are shown in Table II.

In the preceding 3 months, 31.4% of the patients had been admitted to a psychiatric ward; 86.8%, 28.3% and 57.2% was on typical, atypical and depot antipsychotics, respectively. The median (interquartile) expense on these therapies during the period was \$166.40 (\$80.90, \$360.50). At the time of interview, the mean (SD) number of psychiatric symptoms was 4.9 (3.2). Patients' SF-36 scores were substantially worse than Singaporean population norms ($p < 0.05$ for all, one-sample t-tests, Table III).

Factors Influencing Negative Experience of Caregiving

The regression analysis for ECI negative total scores yielded 6 variables, indicating that these factors influenced negative caregiving

TABLE II: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF CAREGIVERS AND PATIENTS

	%, unless otherwise stated	
	Caregiver (n=159)	Patient (n=159)
Mean (SD) age, years	52.4 (14.1)	37.2 (10.8)
Female gender	58.5	50.3
Ethnicity		
Chinese	83.6	84.3
Malay	8.2	10.1
Indian/others	8.2	5.6
Education		
Primary or lower	46.5	30.9
Secondary	45.3	56.5
Tertiary	8.2	12.6
Marital status		
Single	18.2	69.2
Married	59.1	23.9
Divorced/separated/widowed	22.7	6.9
Employment status		
Employed	47.8	10.0
Unemployed	11.3	64.2
Homemaker	40.9	25.8
Presence of chronic medical conditions	46.5	15.1
Survey language (English)	60.4	70.4
Relationship		
Parent	51.6	
Spouse	17.6	
Sibling	17.6	
Child/others	13.2	

TABLE III: PATIENTS' SELF-REPORT HEALTH STATUS

Health status scale	Mean (SD)	Singaporean population norms*
SQLS		
Psychosocial	37.8 (21.1)	
Energy/motivation	46.1 (16.3)	
Symptoms/side effects	27.5 (18.7)	
SF-36		
Physical functioning	74.1 (19.4)	83.9
Role limitations due to physical problems	45.9 (40.8)	84.9
Bodily pain	76.8 (23.4)	81.1 ^a
General health	56.5 (21.5)	68.5
Vitality	54.8 (19.6)	63.4
Social functioning	65.6 (27.4)	76.3
Role limitations due to emotional problems	47.7 (42.9)	82.1
Mental health	58.9 (19.1)	72.5

Scores for all scales range from 0 to 100 (higher SQLS scores indicate worse quality of life/ health status while higher SF-36 scores indicate better health status and functioning); *adjusted for age, gender, ethnicity and survey language (English/ Chinese)¹³; $p < 0.001$ for all unless otherwise specified; ^a $p = 0.023$ (one-sample t-tests);

experience. These factors and their relationships with negative caregiving experience were: caregivers' perceived support from professionals, lower expense on psychiatric therapies and patients' being married, experiencing less psychiatric symptoms, worse mental status or less role limitations due to physical problems were associated with less negative caregiving experience. Effect sizes of these factors are shown in Table IV.

Factors Influencing Positive Experience of Caregiving

The regression analysis for ECI positive total scores yielded 4 variables, indicating that these factors influenced positive caregiving experience. These factors and their relationships with positive caregiving experience were: caregivers' perceived support from family/friends, caregivers' speaking English, patients' recent admission to a psychiatric ward or experiencing more role limitations due to physical problems were associated with more positive caregiving experience. Effect sizes of these factors are shown in Table V.

TABLE IV: EFFECT SIZES OF FACTORS SELECTED BY THE LINEAR MODEL FOR ECI NEGATIVE TOTAL SCORES

	Effect size (unstandardised)	P value
Total expenses on therapies*	0.02	< 0.001
Number of psychiatric symptoms	2.79	0.001
Support from medical professionals* (yes)	-12.28	0.012
SF-36 mental health scores	0.38	0.003
SF-36 role-physical scores	-0.14	0.019
Patients' marital status (married)	-11.35	0.042

Adjusted R²: 24.5%; * in the preceding 3 months.

TABLE V: EFFECT SIZES OF FACTORS SELECTED BY THE LINEAR MODEL FOR ECI POSITIVE TOTAL SCORES

	Effect size (unstandardised)	P value
Support from family and friends (yes)*	3.83	0.015
Patients' admission in a psychiatric ward (yes)*	4.02	0.017
SF-36 role-physical scores	-0.05	0.017
Caregivers' survey language (English)	3.56	0.028

Adjusted R²: 9.7%; * in the preceding 3 months.

Discussion

In this cross-sectional study, we identified a variety of factors that may influence caregiving experience in caregivers of a patient with schizophrenia. To the best of our knowledge, this was the first study with the aim to understand the experience of such caregivers in Singapore.

Some findings in our study are consistent with those in the literature. We found caregivers' self-perceived support was associated with better caregiving experience. Previous studies have shown that self-perceived support from family¹⁴ or professionals¹⁵ was associated with less burden for caregivers of persons with mental disorders. In addition, the observed relationship between patients' psychiatric symptoms and negative caregiving experience in our study has been a replicated finding in the literature.^{16,17}

On the other hand, our finding that patients' worse role functioning was associated with more positive caregiving experience is contrasted with a previous finding that poorer social functioning of patients with severe mental disorders was associated with less positive caregiving experience.¹⁸

In this study, we also found factors of caregiving experience that, to the best of our knowledge, have not been reported in the literature. Some of these new findings may reflect cultural differences in perception of caregiving experience between the West and Singapore. For example, caregivers of married patients perceived less negative caregiving experience in Singapore. Another example is that higher expense on psychiatric therapy was associated with more negative caregiving experience, which may reflect the fact that many of these Singaporean families were from lower socio-economic class.

Limitations of this study include recruiting subjects from only 1 medical centre, which may limit the generalisation of our findings. Another limitation is that only English and Chinese-speaking caregivers and patients were included in the study, as Malay and Tamil versions of the ECI were not available.

Findings in our study, though preliminary, do justify further

studies into those factors that may influence caregiving experience in Singapore. Once these factors and their relationships with caregiving experience are clarified, health professionals may better help these caregivers who play an important role in management of patients with schizophrenia.

Acknowledgement

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A Prediction Model to Identify Risk of Developing Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis in Patients with Erythema Multiforme⁺

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Introduction

Erythema multiforme (EM), Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are part of a spectrum of muco-cutaneous syndromes^{1,2} and are often the result of drug therapies or infections. SJS and TENS have been associated with severe morbidity and mortality of up to 15%³ to 50%.⁴ The aim of this study was to determine predictors for SJS and TEN to allow early recognition of cases likely to have an adverse outcome in patients presenting with EM.

Materials and Methods

All cases presented to the National University Hospital under the International Classification of Diseases Adapted, 9th Modification (ICD-9) coding of 695.1, encompassing EM, SJS and TEN, as a primary or secondary diagnosis were captured electronically. The diagnoses for EM, SJS and TEN were confirmed based on published criteria.^{3, 4, 5}

Data extracted from the case notes included patients' demographics, symptoms on initial presentation, definite and probable causative agents, history of previous drug allergies and existing co-morbidities. A prediction model to differentiate patients with cutaneous rash at risk of developing into SJS or TEN was established based on the above factors. Data were analysed using the Statistical Package for the Social Sciences (SPSS release 10.0, SPSS Inc). Confidence intervals were set at 95%. Classification and Regression Tree (CART version 5.0, Salford Systems) analysis was used to determine the breakpoint for the continuous variable, age and to build a prediction model identifying the order of importance of risk factors for developing SJS or TEN. Significant covariates identified by univariate analysis were then applied to run a stepwise multivariate regression analysis.

Results

A total of 153 cases seen between January 1992 to December 2002 were identified. The casenotes of 89 of these patients could be retrieved for retrospective evaluation. There were 41 (46.1%) SJS/TEN cases and 48 (53.9%) with EMO. All patients classified under SJS or TEN were confirmed to have distinctive skin lesions and involvement of no less than 2 mucous membranes.

All SJS/TEN cases were treated as inpatients or developed drug reactions during hospitalisation. Twelve (25%) EMO cases were treated at the emergency department as outpatients, while the remaining 36 (75%) were hospitalised.

The demographics of the patients are described in Table I. While no statistical significance was shown by chi-square analyses for gender and race distribution between the EMO and SJS/TEN groups, the mean age in the EMO group was significantly lower than the SJS/TEN group; 24.06 ± 22.51 years versus 35.41 ± 25.13 years. The ages of the patients ranged from 10 months to 79 years. CART analysis defined the breakpoint for age as 17.5 years. The binary covariate of ≥18 years was thus used in the subsequent univariate analysis to determine if age group was a predictor for the development of SJS/TEN.

There were 3 deaths based on all cause mortality for these 89 cases. One in the EMO group who died of a non-EM related cause of intracranial haemorrhage and 2 (4.9%) in the SJS/TEN group where multi-organ failure, a secondary complication directly attributed to SJS/TEN, was implicated. The definite causative agents identified in these 2 cases were Anarex®, a combination product of paracetamol with orphenadrine citrate and allopurinol. A total of 11 (from 17 cases) and 18 (from 29 cases) definite causative agents were identified in EMO and SJS/TEN groups, respectively. The definite causative agents for each group are listed in Table II. There were 2 cases each in the EMO group that had skin eruptions attributed to viral infections and food versus none in the SJS/TEN group. Antimicrobials were the most common primary causative agents identified with beta-lactams emerging as the most common class of antibiotics causing skin eruptions. This was followed by NSAIDs in the EMO group [3 (6.25%)] and antiepileptics in the SJS/TEN group [9 (22%)]. Antimicrobials were also identified as a secondary but definite causative agent in another 2 SJS/TEN cases. Antiepileptics were not recorded to be a definite cause for EMO. At least 1 suspected agent was documented in an additional 19 EMO and 26 SJS/TEN cases respectively.

Of the 89 patients, 25 had a history of previous drug allergies, 8 (12.5%) in the EMO group and 17 (41.5%) in the SJS/TEN group. This prompted the use of previous drug allergies to be tested as a predictive covariate for SJS/TEN. The following covariates were tested in separate univariate analyses using the chi-square test to determine their significance – existing co-morbid conditions, age ≥18 years, gender, history of previous drug allergies, fever, pruritus, nausea and vomiting, antiepileptic usage and antimicrobial usage. The significant predictors were existing co-morbidities ($P = 0.001$), history of previous drug allergy ($P = 0.009$) and antiepileptic ($P = 0.001$) or antimicrobial usage ($P = 0.015$) at time of diagnosis. The same covariates were entered into a CART analysis to confirm the importance of their predictive values in the classification tree. The

TABLE I: DEMOGRAPHICS OF STUDY COHORT

Demographics	EM Only	SJS/TEN	P value
Gender			
Male	23	18	0.75
Female	25	23	
Race			
Chinese	36	29	0.67
Malay	7	10	
Indian	1	1	
Others	4	1	
Age			
Mean	24.06 ± 22.51	35.41 ± 25.13	0.027
Admission type			
Inpatient	36	41	0.001
Outpatient	12	0	
Cause for admission			
Drug reaction	17	35	<0.001
Others	31	5	

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⁺Dr Tambyah would like to dedicate this abstract to the memory of Prof Chan Heng Leong, Singapore's and one of the world's foremost authorities on SJS/TEN.

TABLE II: DEFINITIVE CAUSATIVE AGENTS IDENTIFIED

Classes of drugs	EM Group	SJS/TENS Group	Total
Antimicrobials (total)	6	15 ^a	21
Azithromycin	1	0	
Cephalexin	2	2	
Cefuroxime	1	0	
Cotrimoxazole	1	3	
Amoxycillin	1	5	
Cefazolin	0	1	
Penicillin	0	1	
Chloramphenicol	0	1	
Metronidazole	0	1	
Nystatin	0	1	
Antiepileptics	0	9	9
Phenytoin	0	1	
Phenobarbitone	0	2	
Carbamazepine	0	5	
Lamotrigine	0	1	
Xanthine Oxidase Inhibitor (Allopurinol)	0	1	1
NSAIDS ^b	3	3	6
Ibuprofen	3	0	
Naproxen	0	2	
Diclofenac	0	1	
Paracetamol	0	2 ^c	2
Others	4	2	6
Non-drug	4	0	4
Food	2	0	
Viral	2	0	
Unknown	31	12	43
Total	48	44 ^d	92 (89 cases)

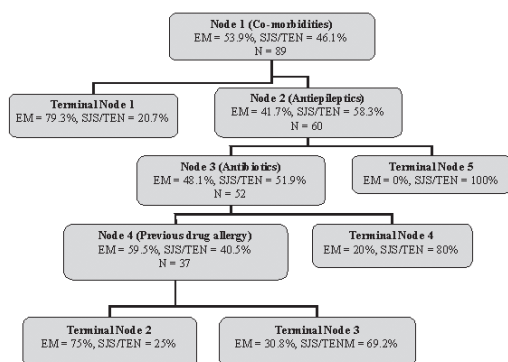
^a 3 cases with 2nd agent implicated^b Non-Steroidal Anti-Inflammatory Drugs^c including 1 case of Anarex(^d 3 cases with 2 agents defined

Fig. 1. Classification tree topology for predicting SJS/TEN from EM.

primary node splitters, in decreasing order of variable importance, were co-morbidities, previous history of drug allergy, antibiotic usage followed by antiepileptic usage. This resulted in a classification tree illustrated in Figure 1, with 5 terminal nodes. The relative cost was 0.525. The learning sample prediction success rate was 85.4% for EMO and 70.7% for SJS/TEN. The corresponding prediction success rates for test samples based on this model were 79.2% and 68.3%.

Other covariates that did not significantly predict risk of developing SJS/TEN included fever, gender, race, age ≥ 18 years and pruritus. An important perspective derived from this study was that mucocutaneous rash when accompanied by co-morbidities, previous history of drug allergies, antiepileptic or antimicrobial usages would predispose a patient to the risk of developing SJS/TEN. The accompanying generic symptoms of fever, nausea and/or vomiting are not indicative of the possibility of developing the more severe forms of EM. Although the mean age of SJS/TEN patients in our study was greater than that in the EMO group, stratification of

TABLE III: UNIVARIATE ANALYSIS OF PREDICTIVE COVARIATES

Covariates	EM only group	SJS/TEN group	P value
Existing co-morbidities*	25	35	0.001
Age = 18 years	26	27	0.263
History of drug allergy	8	17	0.009
Fever	23	24	0.317
Pruritis	19	10	0.127
Nausea, vomiting	2	1	0.653
Antiepileptics	0	9	0.001
Antimicrobials*	6	14 ^a	0.015

^a 1 case had 2 antimicrobials implicated

* by stepwise multivariate logistic regression analysis, these 2 covariates were found to be significantly associated with the development of SJS/TEN from EM

patients into 2 separate age groups using the CART breakpoint of 18 years confirmed that children and adolescents were not more likely to develop severe forms of skin eruptions.

Using a stepwise multivariable logistic regression model, the covariates found to be significantly associated with the development of SJS/TEN from EM were antibiotic usage ($P = 0.005$) and co-morbidities ($P = 0.032$).

Discussion

From the results of this study, 75% of EMO cases and all of SJS/TEN cases were treated as inpatients, accounting for 52 admissions out of 89 cases. Early identification of patients likely to progress from relatively benign cutaneous reactions to severe forms of rash, such as SJS and TEN, is important. From the hospital's perspective, this could potentially lead to a reduction in length of stay and mortality from further complications. TEN in Singapore was well described in a seminal study⁶ published nearly 20 years ago by Prof Chan Heng Leong in the *Journal of the American Academy of Dermatology*. He described 15 patients with TEN, all drug-induced with more than 75% epidermal loss in 7/15 cases. He also reported that the same drug could cause either the benign EMO or TEN. Similar studies have been conducted in the past in Seattle, Washington, comprising 37 cases of EM, SJS and TEN of which 16 were drug-induced;⁵ West Germany, 574 cases of SJS and TEN of which 380 were caused by drugs;⁷ France, 87 TEN cases of which 67 were due to drugs⁸ and Malaysia, 29 cases of EM, SJS and TEN of which 15 were drug-related.⁹ The culprit agents identified in this study, antiepileptics and antimicrobials, were consistent with causative agents in other published studies.^{5,8,10}

Two cases of EM were viral-induced while none of the SJS/TEN cases were linked with infections. *Mycoplasma pneumoniae* and *Herpes simplex* are well-recognised causes of EM and rarely SJS/TEN.¹¹⁻¹³ It was also noted that the SJS and TEN patients had a higher mean age and more had co-morbidities than those in the EMO group. This might not indicate age as a risk factor as these patients might be more exposed to different therapeutic agents due to their age and co-morbidities.

One drawback of this study was its retrospective nature, which limited the collection of detailed drug histories, incubation period, accurate rash descriptions, as well as the performance of skin biopsies to confirm diagnoses. The 10-year period also prevented the comparison of laboratory data.

Conclusions

This study identified 2 groups of drugs — antiepileptics and antimicrobials — as predictors of SJS and TEN. In addition, patients with a previous history of drug allergies and existing co-morbidities also render them as likely candidates for severe forms of cutaneous reactions when they present with drug-related rash. The results of this study suggested that patients with EM, due to these 2 classes of drugs, should be monitored closely for complications such as life-threatening mucocutaneous disorders.

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Best Oral Presentation Award Finalist – Paramedical Disciplines

Chinese Version of Patient Rated Wrist Evaluation (PRWE): Cross-cultural Adaptation and Reliability Evaluation

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Distal radius fracture is a very common injury, it accounts for one sixth of the fractures treated in the emergency room.¹ Despite advancing surgical and rehabilitation management of this injury, deformity due to this injury is not uncommon, and some can lead to permanent disability. Clinical evaluation systems, such as Gartland and Werley point system² and modified clinical scoring system of Green and O'Brien,³ have been widely used as clinical scoring system for evaluating distal radius fracture outcome. In recent years, standardised validated questionnaires and health-related quality of life evaluation have become more important in clinical outcomes measure, such as 36-item Short Form Health Survey (SF-36),⁴ Disability of the Arm, Shoulder, and Hand (DASH)⁵ and Patient Rated Wrist Evaluation (PRWE).⁶ SF-36 is a generic instrument, which allows cross-condition comparison in any population, but may not be sensitive enough to detect small but important changes related to a particular condition. Therefore, region-specific instruments such as DASH and PRWE have been introduced. PRWE is one instrument that is particular to wrist disorders, while DASH is for all upper extremity disorders.

PRWE was widely used in western literature to evaluate wrist disabilities.⁷⁻¹² However, it is not commonly used among Asian population, since there is no validated translated version available. Thus, there is a need to develop a validated Chinese version of PRWE with consideration of the language and culture differences. The present study was to perform a cross-cultural adaptation of the PRWE to Chinese and evaluate the reliability of the Chinese version in patients with distal radius fractures.

Materials and Methods

The Instrument

PRWE is a 15-item questionnaire that equally rates wrist-related pain and disability in functional activities.⁶ It contains 5 questions pertaining to the severity and duration of the pain, which contribute to 50% of the total score. The functional items include 6 specific tasks and 4 questions in which patients rate their disability in the domains of self care, work, household work and recreation. The

functional items contribute to another 50% of the total score. Each item in the questionnaire is scored on a 0 to 10 numeric scale.⁶ The reliability and validity of this assessment tool have been studied in the western population; test-retest reliability was excellent (ICCs > 0.90). Validity assessment demonstrated that the instrument detected significant differences over time ($p < 0.01$).^{6,13} Responsiveness of PRWE, DASH and SF-36 in evaluating recovery after distal radius fracture has been compared; and PRWE was the most responsive questionnaires among the 3.¹⁴

Subjects

Distal radius fracture patients who 1) understand English or Chinese, 2) attend hand rehabilitation program in NUH from January 2002 to December 2002, and 3) had started on wrist mobilisation programme for at least 1 month were included in the study. Exclusion criteria were: age <18 years and/or inability to understand the questionnaire due to cognitive impairment or language difficulties. 21 subjects in the Chinese group (male 13; female 8) and 30 subjects in the English group (male 24; female 6) were recruited for the reliability study.

Adaptation Process

The English version of PRWE was translated into Chinese with conceptual translation by a group of bilingual experienced hand occupational therapists. The translation did not try to achieve "word for word" equivalence, but instead tried to capture the essential meaning of each item in the questionnaire. Face validity was established by introducing a 5-member review panel to critique the Chinese translation, and valid suggestions were taken to improve the translation of the questionnaire.

The version was translated back to English by 2 other bilingual healthcare professionals whose first language was English. Both were blind to the concepts being investigated. The translations were compared with the original PRWE questionnaire. Discussions were held with the translators to jointly "fine-tune" the Chinese translation.

Pilot testing was carried out on 23 patients with distal radius fracture (12 Chinese group; 11 English group) to gather and compare their feedback on suitability and acceptability of both the Chinese

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and PRWE version. The feedbacks were gathered under 4 categories: 1) whether all the statements are easy to understand; 2) whether the activities listed in the questionnaire are relevant to the individual; 3) whether the scale of 0-10 is easy to use; and 4) whether it is fast to complete the questionnaire.

The final Chinese version of the PRWE was then evaluated with regards to reliability and internal consistency.

Reliability Analysis

This study was conducted on patients who sustained distal radius fracture and attended hand therapy programme at NUH. To establish test-retest reliability, the subjects were administered PRWE twice at a week's interval. Either the Chinese or English version of the PRWE was administered, depending on their language preference. Completed PRWE questionnaires were obtained from 51 patients. Test-retest reliability was calculated by computing intraclass correlation coefficient (ICC) for analysis. The ICC analysis was also performed separately for different subtests. Internal consistency of each item within both Chinese and English PRWE was measured by Cronbach's alpha coefficient. The Cronbach's alpha is based on the correlation of each item with the whole scale and the number of items that comprise the scale. The Cronbach's alpha can range from 0-1, and a coefficient of > 0.7 indicates good reliability.

Results

Adaptation

The pilot testing showed that majority of patients agreed that the questionnaires were clear and easy to use and most of the items were relevant to their daily life. The English group had slightly better feedback compared to the Chinese group (Table I).

Reliability

Test-retest reliability analysis is shown in Table II. The ICC for the Chinese version was 0.93 comparable to the English version of 0.94 in the current study, indicating excellent agreement between the scores from the test and retest. The different subtests for the Chinese version also showed high ICC, which ranges from 0.88-0.91.

Results of internal consistency are shown in Table III. Cronbach alpha was > 0.7 for all scales, which ranges from 0.94-0.98 for the Chinese version, indicating high correlation between each item and the whole scale.

Discussion

There has been an increase in research that evaluates clinical measures that are translated and adapted for different cultures.¹⁵ The benefit of culturally adapted instruments is that it allows comparison of outcome between countries, and utilising validated instruments from others saves the effort of reproducing the instruments. However, the social-cultural factors and cultural differences may influence the performance of a questionnaire and the results interpretation. For instance, during the adaptation process, one of the items in the specific activities category, "cut meat using a knife in my affected hand" was originally referring to a feeding task. But using knife for feeding is not a common practice in Chinese culture.

TABLE I: RESULT OF PILOT TESTING (% of subjects who agreed with statements)

	Easy to understand	Activity relevance	Easy to use scale	Fast to complete
Chinese group (n=12)	75%	92%	54%	42%
English group (n=11)	100%	82%	82%	100%

TABLE II: TEST-RETEST RELIABILITY

	Chinese version (n = 21) ICC	English version (n = 30) ICC
Pain	0.91	0.90
Specific activities	0.90	0.93
Usual activities	0.88	0.92
Total score	0.93	0.94

TABLE III: INTERNAL CONSISTENCY

	Chinese version (n = 42) Cronbach alpha	English version (n = 60) Cronbach alpha
Pain	0.94	0.93
Specific activities	0.94	0.96
Usual activities	0.97	0.92
Total score	0.98	0.98

In this study, Chinese adaptation of the PRWE was performed using a systematic standardised approach. Similar approaches were performed in the adaptation of other health instruments, such as the reliability and validity study of DASH Swedish version,¹⁶ and the validation of Arthritis Impact Measurement Scale Spanish format.¹⁷ The studies proceeded according to established guidelines: using focus group for quality control, back translation and pilot testing.

The present study produced the Chinese version of the PRWE, and the results showed high test-retest reliability and internal consistency. In this study, 1 week interval was chosen to minimise the patient's recall of the previous answers. If the interval is too long, patients may experience functional change due to the natural recovery process.

Our finding shows excellent reliability and internal consistency of the Chinese version of the PRWE. It should be a valuable tool in clinical research in wrist disorders and for clinicians to evaluate treatment outcome.

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Osteogenic Role of Vascular Endothelial Growth Factor in Bone Regeneration

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Introduction

Many *in vitro* studies have shown that VEGF was able to stimulate osteogenesis apart from angiogenesis.¹ Deckers et al¹ suggested that vascular endothelial growth factor (VEGF) play an important role in the regulation of bone remodeling by attracting endothelial cells and osteoclasts and by stimulating osteoblast differentiation. The relationship between angiogenesis and osteogenesis was investigated by Rabie² and found to be closely related.

Currently, very few *in vivo* studies on the osteogenic capability of VEGF were done. Street et al³ used the rabbit model to apply VEGF locally using a pump to the site of bone damage and found that VEGF does promote new bone formation. They concluded that it might be an effective therapy to promote human bone repair.

Recently, orthopaedic tissue engineering strategies focused on the use of scaffolds as a delivery system for growth factors.⁴ Poly- α -caprolactone (PCL) is one of the synthetic polymers used as scaffold for bone regeneration which is non-toxic, biocompatible and highly compatible with osteoblast. It has a slow degradation rate which is ideal for load bearing applications in bone regeneration. It can be made into three-dimensional porous scaffolds and such a patent has been filed.⁵

This study was designed to investigate the osteogenic capability of VEGF in bone defects using fibrin glue as carrier and the fused deposition modelling (FDM) porous PCL as scaffold.

Materials and Methods

Thirty-two male adult New Zealand White rabbits (3.5- 4kg) were used in this study.

The surgical procedure involved an anteroposterior midline skin incision over the cranial vault of about 4 to 5 cm. Defects of 10 mm diameter were created in the parietal skulls of the rabbits using a trephine bur.

A total of 40 defects were created and randomly divided into 4 treatment groups. Twelve defects in 12 rabbits were grafted with PCL impregnated with rhVEGF (Calbiochem) and fibrin glue (Tisseel®, Immuno AG) as VEGF/ Fibrin/ PCL groups. Another 12 defects in 12 rabbits were grafted with PCL impregnated with fibrin glue only as Fibrin/ PCL groups (active control). Sixteen defects in 8 rabbits were used for the last 2 groups of control. Eight defects were grafted with PCL (active control) and the other 8 defects were left empty (passive control). All wounds were closed with interrupted 3/0 black silk sutures.

The rabbits were sacrificed and the tissue specimens were harvested at 1 week and 2 weeks (equal numbers from each group) for histological analysis. The specimens were fixed in formaldehyde, decalcified in 20% ethylene-diamine tetraacetic acid pH 7.2 (EDTA) and embedded in paraffin wax. Four sections of 5 microns were randomly selected from each specimen, sectioned and stained in haematoxylin and eosin. The amount of new bone formed was quantitatively and qualitatively analysed using the Olympus Micro Image software.

Ultra structural identification of cells involved in the healing of the experimental group, VEGF/ Fibrin/ PCL at 2 weeks, was also carried out. Tissues were harvested, fixed in glutaraldehyde, demineralised with 20% EDTA, postfixed in osmium tetroxide (OsO₄) and trimmed into 1 mm blocks and embedded in resin. The cells are identified using transmission electron microscope (TEM).

Data were analysed with statistical analysis package Statistical Package for Social Science (SPSS) Version 11.0. A preliminary two-way analysis of variance (Two-way ANOVA) was performed. Post hoc comparisons between groups were performed using Tukey HSD test. Student's t- test was used to compare the 1 week and 2 weeks groups. Level of statistical significance was set at $p < 0.05$.

Results

Quantitative

The amount of new bone formed for all groups is shown in Figure 1. Significantly more new bone was formed in both the groups grafted with VEGF/Fibrin/PCL compared to the control groups ($p < 0.01$). The amount of bone was found to be 352% ($p < 0.01$) higher in the 2 weeks VEGF/ Fibrin/ PCL group compared to the corresponding 1 week group. The amount of bone formed in the fibrin/PCL group was significantly more than that formed in the PCL and empty groups ($p < 0.01$).

Qualitative

Histologically, osteoid could be seen surrounded by osteoblasts at the frontal region of the newly regenerated bone, with osteocytes embedded in them (Fig. 2). Blood vessels were present in abundance in the surrounding area.

Mesenchymal cells, preosteoblasts, osteoblasts and osteocytes were identified under TEM. New bone formation was observed next to host bone. Numerous small blood vessels were seen in close proximity with the new bone formed.

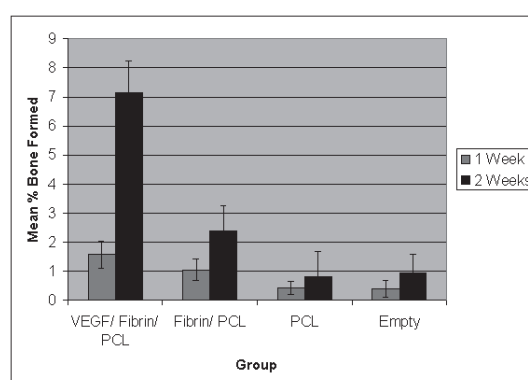


Fig. 1. Graph showing mean percentage bone formed.

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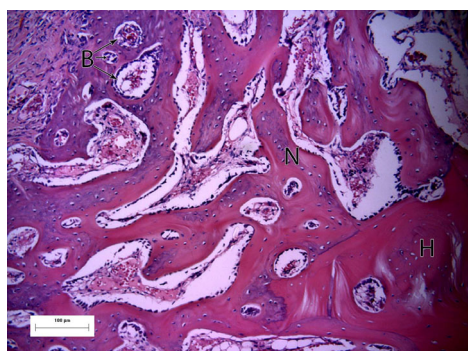


Fig. 2. VEGF/Fibrin/PCL at 2 weeks showing new bone (N), host bone (H) and blood vessels (B).

Discussion

Our results demonstrated that VEGF enhances early osteogenesis *in vivo* which concurred with the findings of Street et al.³ This is largely due to the angiogenic property of VEGF which is probably one of the most critical important factors for successful osteogenesis.^{2,3} As shown by histological analysis, numerous blood vessels were detected at the site of the new bone formation. In this study, as only a single dose of VEGF was applied locally to the site of defect, its effect was expected to be short-lived. Therefore, this study was designed to show the short-term effect of VEGF delivered by fibrin in PCL scaffold. For future studies into the long-term effect of VEGF, incorporation of the growth factor into the PCL scaffold would be the most ideal, as the release of VEGF will be sustained longer as PCL degrades slowly.

The ultrastructural identification of cells involved in the healing of the cranial bone defects revealed a normal healing pattern with a route of entry for preosteoblasts and secretory osteoblasts surrounding the new osteoid bone situated nearby to the host bone. This demonstrated active osteogenesis during early stage of bone regeneration.

Fibrin was used in this study to improve the handling of the VEGF, as VEGF is reconstituted in solution form. Fibrin may have slight osteogenic properties by itself and can provide an excellent fibrogenic network to enhance osteoblasts mobility when associated with a growth factor.⁶ Its biocompatibility makes it a good healing medium. In our study, fibrin glue could support the osteogenic activity of VEGF as shown by significantly more bone formed in the Tissue/PCL group compared to the PCL and empty groups.

PCL is a porous scaffold which serves as a three-dimensional template for initial cell attachment and subsequent bone tissue

formation. It is appropriate in this study as it serves as a resilient material for delivery of VEGF for bone regeneration. The PCL scaffold used in this study was fabricated in the Laboratory of Biomedical Engineering, BIOMAT, National University of Singapore (in collaboration with Temasek Polytechnic) by the FDM scaffold fabrication process. The FDM process forms three-dimensional objects from a CAD file as well as digital data produced by an imaging source such as computer tomography (CT) or magnetic resonance imaging (MRI). The process draws the designed model (scaffold) one layer at a time. This allows scaffolds of different micro-architectures (for example, porosity, wall thickness) to be custom made to requirements.

This study suggests that VEGF delivered by fibrin glue in PCL scaffold may be useful to promote osteogenesis in bone repair. This gives rise to many potential clinical applications. In orthodontics, these applications may include jaw reconstruction and repair of alveolar bone defects caused by trauma, pathology, clefts, early loss of teeth, periodontal disease or even loss of buccal plate of bone during extraction of buccally placed teeth.

Conclusion

VEGF delivered by fibrin glue in PCL scaffold does promote osteogenesis in early stage of bone regeneration and may be used for its osteogenic role in bone repair/ reconstruction application.

Acknowledgement

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Evaluation of Hemispheric Language Dominance in Adult Craniopagus Twins with Functional Magnetic Resonance Imaging (fMRI)

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Introduction

Conjoined Siamese twins are uncommon, with an estimated incidence of between 1:50,000 to 1:80,000 births. Of these, cranially conjoined or craniopagus twins are even rarer, with an incidence of 1 in 2.5 million births.¹⁻³ The evaluation of craniopagus twins, particularly in the adult setting, poses unique challenges to the attending surgeons and radiologists. Imaging to accurately define the anatomic fusion, the morphology of the underlying structures, including the vascular anatomy is important to guide therapy decisions, for surgical planning and prognostic information.

Apart from structural-morphologic evaluation, it is important to understand and define the underlying functional status of the brain pre-operatively. In particular, determination of the functionally important or eloquent brain areas controlling language function and the cerebral hemispheric language dominance is desired. Defining the eloquent language areas will help guide the operative approach should surgery be employed, and minimise morbidity. Such information may be obtained non-invasively by functional magnetic resonance imaging (fMRI) techniques using the blood oxygen level dependent (BOLD) effect. We present our experience in the fMRI evaluation of adult craniopagus twins to map the eloquent language areas during pre-surgical evaluation. To our knowledge, this is the first report in the medical literature of fMRI in craniopagus twins.

Materials and Methods

Patients

27-year-old female twins with parieto-occipito-temporal craniopagus were evaluated. Both had graduated from university, were intellectually normal and fluent in Persian (first language) and English. The right twin (A) was right-handed while the left twin (B) was left-handed. Structural-morphologic information had been obtained from computed tomography (CT), MRI and angiogram studies done in another centre in Singapore and previously in Germany.⁴ These indicated a partially shared cranium with the cerebral and cerebellar hemisphere components present in both twins, with their temporal and parietal lobes on the common fused side compressed and deformed. There was a common, shared superior sagittal sinus posteriorly.

fMRI Tasks

Four cognitive tasks were presented to each twin individually and in the same order consecutively. First was a visual perception task that required looking at a flashing checkerboard sequence. Significant fMRI BOLD activation was expected in the bilateral primary visual and visual association areas in this control task. Next was an English reading task in which each twin was required to silently read simple English words flashed one at each time. Such a task was expected to elicit activations at the major language areas of the brain, ie, Wernicke's area and Broca's area (or their homologous counterparts)

in addition to visual areas. The third task was an English picture-naming task, where each twin was required to silently name in English each object that appeared on the screen (adapted from Snodgrass & Vanderwart, 1980).⁵ Lastly, a Persian picture-naming task was presented, where each twin silently named in Persian the objects shown. These two tasks were expected to elicit bilateral prefrontal areas in addition to the areas mentioned for the other tasks. The computer-generated stimuli were presented via fiberoptic cables to binocular goggles that were adapted for each twin. Stimuli were designed and controlled by MacStim software.

Data Acquisition and Analysis

fMRI data was acquired on a 1.5T MRI scanner (NVi, GE Medical Systems, Wisconsin, MI) using a continuous T2*-weighted gradient-echo EPI sequence (per task 60 volumes with 8 axial slices each; TR: 2000 ms; TE: 40 ms; 8 mm slice thickness; FOV: 35 x 35 cm; 128 by 128 image matrix). Pre-processing and analysis of the data were done primarily using SPM99, based on the general linear model. Data were realigned, corrected for slice-timing and spatially smoothed with a Gaussian kernel of 14 mm FWHM. Data were modelled using a box-car function that was convolved with the haemodynamic response function (HRF) and a temporal derivative, which allows for the modelling of slight onset differences of around a second. Temporal smoothing of the data and design matrices was performed using a high-pass filter of 1/80 s and a low-pass filter using a Gaussian kernel of 4 mm FWHM. We reported activation if there were clusters of 3 or more voxels above a threshold of $p < 0.005$, uncorrected for multiple comparisons. Clusters significant at this level and which were activations expected due to apriori anatomical hypotheses were further evaluated statistically using a small volume correction.⁶ Results were independently verified using Brain Voyager v 4.9 software. The cerebral hemispheric dominance of task activation for each twin was calculated using the Laterality Index (L-R)/(L+R). Language dominance was defined as > 0.2 , as reported in other studies.⁷⁻¹⁰ Broad and homologous brain regions in each hemisphere, including regions around Wernicke's area and Broca's area, were defined.

Results

The visual perception task elicited significant BOLD activation in both the twins in the primary visual area. The visual association areas (precuneus, cuneus) were additionally activated in Twin A. The English reading task activated the language processing areas in the left cerebral hemisphere, namely the left middle temporal lobe around Wernicke's area and the inferior frontal cortex around Broca's area.

In Twin A, the picture-naming task in English activated the left inferior and middle frontal gyrus, bilateral superior and middle frontal areas, the left middle temporal areas, the left postero-inferior

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parietal cortex and visual areas. Twin B showed interesting activation in the left medial, superior temporal and the left inferior, middle temporal/occipital areas.

The Persian picture-naming task activated the left middle temporal area and the left superior frontal area in Twin A, while Twin B showed activation in the bilateral superior frontal and right middle temporal/occipital area.

Results were in accordance with normal data, but locating the activations was made more difficult by the complex neuroanatomy. Both twins were left hemisphere dominant for language function.

Conclusion

Non-invasive language mapping using fMRI has been demonstrated for the first time in total craniopagus. Conventional imaging equipment originally designed for single subjects was successfully adapted and tailored for the twins to permit adequate scan coverage of a larger region comprising 2 adult brains, while maintaining the diagnostic quality of the image data. Compared to our routine protocols, there was a relatively low signal-to-noise ratio (SNR) due to the scanning parameters, as we had to adapt towards a reasonable duration of scanning, to minimise head movement which will degrade image quality and affect data interpretation. Indeed, some head movement artifacts were encountered in the latter part of the fMRI scans for twin B. This diminished the ability to detect task-related BOLD responses and made it difficult to pinpoint activations due to the complex neuroanatomy. Nonetheless, as we performed a robust panel of fMRI tasks, there was sufficient data for analysis and conclusion. There was primarily left lateralisation of language function in both twins despite differing handedness.

fMRI is a useful non-invasive test for determination of eloquent brain areas controlling vital functions such as language, motor function and vision; and has been used routinely for pre-surgical planning and guidance in brain tumour surgery and epilepsy surgery. It offers a one-stop imaging solution whereby structural MRI scans are obtained together with functional data during the same scan session, and image fusion subsequently performed. Previously, determination of vital brain regions controlling various functions had to be performed with intra-operative electrode stimulation with the patient typically awake. For determination of language hemispheric

dominance, an invasive WADA test requiring the intra-arterial injection of sodium amytal during a catheter cerebral angiogram was previously routine. WADA testing has largely been supplanted by fMRI when the latter is available.

Our fMRI results for the twins are in accordance with the results of an independent WADA test performed at a separate examination (unpublished data – Lim SH et al, 2003). Indeed, the vast majority of normal subjects are left hemisphere dominant for language function, regardless of whether they are right or left handed.¹¹ Determination of the eloquent brain areas pre-operatively will help guide therapy decisions, selection of the best approach should surgery be contemplated and offer prognostic information.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry

BRCA1 c.2845insA is a Founder Mutation in Singaporean Malay Women with Early Onset Breast/Ovarian Cancer

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Introduction

Breast cancer is the most common cancer in Singaporean women. The incidence rate in 1993-1997 was 2.3 times higher than in 1968-1972, with an annual increase significantly higher in premenopausal

than postmenopausal women.¹ Of note, the Singaporean Malay ethnic group has shown the greatest increase in the incidence of breast cancer (4.4%) over the past 3 decades when compared to the Chinese and Indian subpopulations.

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Approximately 5% to 10% of all breast and ovarian cancer cases are believed to be attributable to the inheritance of germline mutations in high-penetrance, autosomal dominant susceptibility genes. The identification of one such gene, *BRCA1*,^{2,3} has rapidly led to the characterisation of mutations in this gene among high-risk families, as well as early-onset breast and/or ovarian cancer patients or tumour series in populations worldwide. Several founder and recurrent mutations have now been well documented in specific ethnic groups, such as the Ashkenazi Jews, and in Caucasian populations.⁴ Studies in Asian populations, though on the increase, have been few and amongst these Asian populations, recurrent and founder mutations have now been reported for the Japanese, Filipinos, Thais and Chinese.⁵⁻⁸

Given the increase in the incidence of breast cancer together with the rise in the number of patients presenting with early-onset breast cancer amongst the Singaporean Malays, we have characterised the germline *BRCA1* mutations in patients presenting with early-onset breast or ovarian cancer in order to evaluate the proportion of cancers attributable to mutations in this gene.

Materials and Methods

Study Patients

From 2001 to 2003, 72 consecutive unrelated Malay patients

TABLE I: *BRCA1* SEQUENCE ALTERATIONS IN SINGAPORE MALAY PATIENTS WITH EARLY ONSET BREAST/OVARIAN CANCER

Case	Exon	Sequence alteration	Amino acid change	Age (y)	Family history breast/ovarian cancer
<i>BRCA1</i> sequence variants					
Frameshift mutations					
N536 (Br)	11	c.2845insA	N909fsX5	35	Br33 (S)
N587 (Br)	11	c.2845insA	N909fsX5	44	Br60 (M), Br50 (MA)
N610 (Br)	11	c.2845insA	N909fsX5	35	Ov49 (M)
N633 (Br)	11	c.2845insA	N909fsX5	44	YTD
N521 (Ov)	11	c.2845insA	N909fsX5	49	Br37 (S), Br30 (MA), Br40 (MC)
N576 (Ov)	11	c.2845insA	N909fsX5	50	Nil
N590 (Ov)	11	c.2845insA	N909fsX5	40	Nil
Missense mutations					
N591 (Br)	6	c.335C>A	S72R	41	Nil
N536 (Br)	10	c.778C>A	S220Y	35	Br33 (S)
Neutral polymorphisms					
N33 (Br)	3	233G>A	K38K	38	Nil
N522 (Br)	3	233G>A	K38K	32	Nil
N599 (Br)	1	c.5106-68A>G	IVS16-68A>G	29	Nil
N616 (Br)	17	c.5106-68A>G	IVS16-68A>G	35	Nil
N599 (Br)	17	c.5106-68A>G	IVS16-92A>G	29	Nil
N616 (Br)	17	c.5106-68A>G	IVS16-92A>G	35	Nil
Intronic variants					
N591 (Br)	6	c.420+26CA>AC	IVS6+26CA>AC	41	Nil
N591 (Br)	6	c.432-44G>A	IVS5-44G>A	41	Nil
N511 (Br)	8	c.561-34T>C	IVS7-34T>C	34	Nil
N587 (Br)	18	c.5272+72G>C	IVS18+72G>C	44	Br60 (M), Br50 (MA)

ins: insertion; YTD: yet to be determined

Cancer site: Br = breast, Ov = ovary

Affected relatives: mother (M), maternal aunt (MA), maternal cousin (MC), sister (S)

TABLE II: GENOTYPES FOR THE *BRCA1* c.2845insA MUTATION IDENTIFIED IN 6 PATIENTS

Case	Intron 12 D17S1323	Intron 19 D17S1322	Intron 20 D17S855
N536 (Br)	155 159	119 127	142 148
N587 (Br)	157 159	115 127	142 142
N610 (Br)	153 159	119 127	142 146
N521 (Ov)	153 159	119 127	142 146
N576 (Ov)	157 159	121 127	142 144
N590 (Ov)	153 159	125 127	142 148

Common haplotypes are shown in **bold**

presenting to the National University Hospital were eligible for *BRCA1* testing if they had a diagnosis of breast or ovarian cancer before the age of 45 years and 50 years respectively. In addition, blood samples were collected from 114 unrelated normal subjects randomly selected from the general population. This control set was matched to the cases on the basis of race, gender and 5-year age intervals.

Molecular Studies

DNA isolation and mutation analysis of *BRCA1*

Total genomic DNA was extracted from the peripheral blood lymphocytes using standard procedures.⁹ The entire coding sequence, splice junctions and neighbouring intronic regions of *BRCA1* were screened using a combination of methods primarily single-strand conformation polymorphism (SSCP)¹⁰ and the protein truncation test (PTT).¹¹ All PCR products showing an electrophoretic variant pattern were directly sequenced. The deleterious mutations and genetic variants were named according to the practice of the BIC database,^{10,12} and any insertions or deletions were designated according to HUGO recommendations.¹³

Haplotype analysis

The disease-associated haplotypes in all the patients with the recurrent mutation and 76 controls were constructed by genotyping three intragenic *BRCA1* microsatellite markers D17S855, D17S1322 and D17S1323.^{14,15}

Results

In the 72 patients with early-onset breast or ovarian cancer under the ages of 45 years and 50 years respectively, 10 distinct *BRCA1* sequence alterations were identified in 14 patients.

The frameshift mutation, which produces a truncated protein signal at codon 914, was found in 7 apparently unrelated patients (Table I). Four of the 7 cases (57.1%) had at least a first or second-degree relative with breast or ovarian cancer. Three patients were early-onset breast cancer patients with positive family history of breast/ovarian cancer whilst the other 3 were ovarian cancer patients, of which only N521 reported a family history of breast carcinoma. The family history of patient N633 was yet to be determined at the time of publication. *BRCA1*c.2845insA was first reported in a retrospective study of apparently sporadic breast cancer patients¹⁰ and subsequently described in another study where it was found in 2 of 7 unrelated subjects of Malay ethnicity.¹⁶ None of the 114 unaffected controls were shown to harbour this mutation.

The genotypes at 3 *BRCA1* intragenic markers were examined for 6 unrelated affected patients with the mutation (Table II). No relatives were available to establish linkage phase, but for each of the loci, 1 allele was present in all 6 mutation carriers. For D17S1323 and D17S855, this allele was also common in the control individuals; but for D17S1322, this allele was only found twice in 152 chromosomes (1.3%). These data are consistent with all the c.2845insA mutations being found on a chromosome with a common haplotype, supporting an ancestral link. None of 76 unaffected controls shared the same haplotype as the 6 positive samples.

Two missense mutations of unknown significance, c.335C>A (S72R) and c.778C>A (S220Y), were detected.

Three neutral polymorphisms were detected in this study. Two patients harboured the single base substitution, c.233G>A; whereas the same 2 intronic polymorphisms, IVS16-68A>G and IVS16-92A>G, were found in 2 other breast cancer patients. These 3 polymorphisms have been reported previously in the BIC database and they are among the top 20 mutations frequencies that have been described by designation.

Discussion

Studies carried out in the Singapore population by our group and others have shown a similar prevalence of *BRCA1* mutations as has been seen in reports from western series.^{16,17} Interestingly, one common finding was that the same *BRCA1* deleterious mutation 2845insA was found in 2/7 Malay patients in one study suggesting the presence of a founder mutation.¹⁶ Follow-on studies confirming such a possibility, combined with the low incidence of mutations elsewhere in the gene, would have more significant clinical applications as a simplified mutation test limited to specific regions of the gene would allow for more widespread gene testing, and a more accurate estimate of mutation penetrance outside of multiple cancer families.

To test the above hypothesis, we have studied the spectrum of *BRCA1* mutations in 72 Singaporean Malay patients with early onset breast/ovarian cancer. The prevalence of *BRCA1* mutation in Southeast Asian breast and ovarian cancer patients, in particular of Malay ethnicity, has not been adequately reported and our study constitutes the largest cohort of such Malay patients to date. In this study, the prevalence of *BRCA1* mutation carriers in Malays was found to be similar to that of outbred populations and despite a full analysis of the entire coding region and adjacent intronic segments, all the deleterious mutations were found to be c.2845insA. In addition, none of the controls were found to carry this mutation. To the best of our knowledge, all reported *BRCA1* mutations in Malays have been shown to occur at this position with the exception of the c.5448insC mutation.¹⁸ Haplotype analysis of 6 out of the 7 patients with the recurrent c.2845insA mutation showed that shared haplotype was seen for all 3 intragenic polymorphic markers, strongly suggesting the presence of a founder effect at this locus. Interestingly, this haplotype was not seen in 76 controls which were analysed indicating that this may be a specific haplotype that exists within a specific subpopulation in the Malay population and that this haplotype is associated with increased susceptibility to breast/ovarian cancer.

Although *BRCA1* mutations have largely been reported to occur throughout the gene, recurrent and founder mutations have now been identified in specific subpopulations which have historically been geographically or politically isolated from surrounding populations. The present-day Singaporean Malays are part of a race of people inhabiting what is today Peninsula Malaysia and portions of adjacent islands of Southeast Asia, including the east coast of Sumatra, the coast of Borneo, and the smaller islands that lie between these areas, including Singapore. The Malays are typically characterised by their ethnically homogenous structure and low propensity towards immigration. In addition, the absence of the carrier haplotype among the 76 controls in our study also raises the possibility of a distinct sub-group at increased risk among the Malays.

These results have significant clinical applications as a directed search for the founder mutation is likely to account for the majority of mutation carriers in the population. Simplification of the mutation screening process will allow for a more widespread gene testing outside of hereditary breast and/or ovarian cancer syndromes, which

are rare in our general population. In addition, the identification of carriers outside of these families will also allow for a more accurate assessment of the penetrance of *BRCA* mutations, a vital requirement for genetic counselling and clinical decision-making regarding intervention, with either closer surveillance or prophylactic surgery.

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The Effect of Mulligan Traction Straight Leg Raise in Participants with Short Hamstrings

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Introduction

Background

Hamstring strains are a common athletic injury. Increasing hamstring flexibility is a common goal for athletes, coaches and sports medicine clinicians. Appropriate muscle flexibility assists in preventing or minimising injuries, as well as enhancing performance.

The straight leg raise (SLR) test is used to assess hamstring length. Flexibility is represented by the angular measurements of unilateral hip flexion with the knee in full extension.

Manual techniques developed by Brian Mulligan are used in clinical settings worldwide. The Mulligan Traction Straight Leg Raise (MTSLR) technique is a single, painless intervention with immediate effect. This technique has not been investigated in terms of its effectiveness in improving SLR range of motion (ROM) or the duration of improvement in a population with short hamstrings.

Objectives

To determine if the MTSLR technique can improve SLR ROM in subjects with short hamstrings and if any improvement is maintained 24 hours after the intervention. Secondly, to determine whether any improvement is due to a change in hip flexion or posterior pelvic rotation.

Materials and Methods

Study Design

A prospective, randomised, double blind, placebo-controlled 24-hour follow-up intervention study design was used.

Participants

Forty-seven volunteers were recruited. Participants were required to be 18-40 years old and have a first onset of a stretching sensation in the posterior thigh at <SLR 70°. Participants were excluded if they had a history of hamstring strain, low back pain with referral into the leg, SLR limited by symptoms within or distal to the popliteal fossa, unable to achieve full knee extension or SLR <40°. Participants were randomised to either a MTSLR (n = 24) or placebo group (n = 23). The mean age was 23 years (SD 5.7) for intervention and 21.4 years (SD 3.0) for placebo group. The mean SLR ROM was 54.8° (SD 11.1) for intervention and 54.7° (SD 11.5) for placebo group. There were no significant differences between both groups for age ($P = 0.249$) or SLR ROM ($P = 0.968$).

A sample of 23 participants in each group was required to provide an 80% power to detect a 12-degree change. This change was selected to represent a clinically significant increase in SLR based on the effect size demonstrated by a study of a normal population.

Three participants from each group were lost to follow-up. An independent sample t-test showed no significant differences in the dependent variables of these six participants ($P > 0.05$). One participant was withdrawn from the study after he complained of pins and needles behind his knee during the intervention.

The participants indicated which technique they thought they had received to assess the effectiveness of the blinding procedure. There was no significant difference in the number of participants who perceived they had received MTSLR in the intervention (n = 12) and placebo (n = 8) groups ($X^2 = 1.6$; $df = 1$, 40; $P = 0.343$).

Equipment

Participants were fitted with an ankle-foot orthosis (AFO) and a knee extension splint. Two plinths were placed together, with a board in between. The participant lay supine with one leg on either side of the board. A 10-cm bar was fixed to the AFO at the heel. This bar extended medially and kept in contact with the board to ensure the limb remains in the sagittal plane during SLR measurements.

SLR ROM was measured using two bubble inclinometers. One inclinometer was attached to the pelvis to measure posterior pelvic rotation. The second was attached to the knee to measure SLR ROM. Hip flexion range was calculated by subtracting the pelvic rotation range from the SLR ROM.

The participants were instructed to allow their limb to be raised passively and to indicate the point at which they felt the first onset of stretch. The SLR was performed four times to minimise any increase attributed to the repetitive stretch of the muscle-tendon unit. Measurements were recorded on the fifth, sixth and seventh repetitions. One investigator raised the limb throughout the investigation, while another recorded all inclinometer readings. These investigators were blinded to the participants' group allocation.

A reliability study was performed on 7 participants independent of the study. The intra-session intra-class correlation coefficient (ICC_{3,3}) was 0.99 ($P < 0.001$) for total SLR range and 0.99 ($P < 0.001$) for pelvic rotation range. The inter-session ICC_{3,3} was 0.99 ($P < 0.001$) for total SLR range and 0.99 ($P < 0.001$) for pelvic rotation range.

Procedure

The MTSLR and placebo techniques were performed in a separate room by a third investigator. The leg was raised into flexion until the participant noted the onset of stretch. The MTSLR involved applying a sustained traction to the limb followed by internal or external rotation, or abduction or adduction. If the stretch was decreased, the limb was raised further into flexion. The placebo technique involved SLR until the onset of stretch followed by a calf squeeze.

Data Analysis

The datum was analysed using SPSS version 11.0. One-sample Kolmogorov-Smirnov test was used to establish normality. Two-way ANOVA test was used to analyse the datum with group and testing time as the independent variables and SLR ROM as the dependent variable. One-way repeated measures ANOVAs were performed for each group in order to identify whether any change in SLR overtesting time was due to hip flexion or posterior pelvic rotation ROM. The alpha value was set at 0.05.

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Results

There was a significant increase in SLR across testing times. Tests of within subject contrasts showed a significant increase in SLR ROM between pre-test and post-test ($P < 0.001$), and between pre-test and follow-up ($P = 0.003$). The mean increase in SLR ROM at post-test was 6.3° (SD 5.98°) for the intervention and 4.7° (SD 4.97°) for the placebo group. However, this increase was not significantly different between both groups ($P = 0.349$).

Posterior pelvic rotation was significantly increased at post-test in both intervention ($P = 0.001$) and placebo ($P = 0.001$) groups. This was maintained at follow-up in both intervention ($P = 0.028$) and placebo ($P = 0.007$) groups. Hip flexion was significantly increased at post-test in the intervention group ($P = 0.014$), but not the placebo group ($P = 0.099$). Hip flexion was not significantly different at follow-up in the intervention ($P = 0.945$) or placebo ($P = 0.455$) group.

Discussion

The increase in SLR ROM in the intervention group at post-test was due to an increase in both hip flexion and posterior pelvic rotation, whereas the increase in the placebo group was due only to posterior pelvic rotation. This suggests that the MTSLR had an effect on hamstring flexibility; however, the magnitude (hip flexion increase $\sim 3^\circ$) is not clinically relevant.

The significant increase in SLR ROM in both groups is unlikely to be due to measurement error as intra- and inter-session measures were found to be highly reliable ($ICC_{3,3} = 0.99$).

The large number of SLRs that were performed during testing was designed to eliminate the viscoelastic properties of muscle. It is unlikely that the increase in range was due to the repeated SLR application, as all SLRs were limited to the first subjective onset of stretch and were maintained for only a few seconds for recording measurements.

The measurements may have been influenced by the participants' anxiousness about receiving a novel technique. Participants were informed of the aims of the study through informed consent and, therefore, expected an increase in SLR ROM as a result of intervention. This may have led to an expectation-conditioning adjustment. A

Pavlovian response is unlikely to explain the results as the intervention was only applied once. This study was unable to demonstrate the true magnitude of any placebo effect as the participants were effectively blinded to their group allocation.

There was difficulty controlling participants' athletic activity prior to recruitment. SLR ROM may have appeared higher due to the presence of post-exercise muscle stiffness at initial testing. Recruiting participants 24 hours before initial testing may have controlled this confounding effect.

A study on a normal population found a significant increase in SLR ROM (13.3°) following the application of the MTSLR technique, which was greater than the 6.3° observed in the current study. The normal population study may have experimenter expectancy bias and good subject effect, as it did not have a placebo or control group. The investigators also did not perform any warm-up SLRs before measurements.

Clinical Implications

Even though the improvement in SLR through the application of MTSLR may be related to a placebo effect, it may still be an effective technique. Future research should investigate the MTSLR in comparison to a no-intervention control group using a single-blind study design with blind measurers to effectively evaluate the placebo effect.

The large standard deviation in SLR increase in the intervention group at post-test and follow-up indicates that MTSLR had a large effect on some participants. Research should be directed at further defining the appropriate population that MTSLR should be applied to. Additional research could also investigate MTSLR in comparison to other stretching techniques, as a multiple intervention, or in a population with abnormal neurodynamics.

Conclusion

This study demonstrated that the MTSLR technique does not improve SLR ROM more than a placebo technique in a population with short hamstrings. Both groups had a significant increase in SLR ROM at post-test and follow-up measures; however, it is questionable whether the small increases are clinically significant.

Prevalence, Awareness and Control of Hypertension in Community-dwelling Elderly in Singapore

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Introduction

The prevalence of hypertension in the elderly has been quoted to range from 43% to 65% in Asia^{1,2} and some parts of Europe.^{3,4} In the National Health Survey 1998,⁵ prevalence of hypertension in Singapore was 27.3% in the 30-69 years' age group, but a very high 64.3% in the 60-69 years' age group. Amongst this high prevalence is another issue of awareness: up to 53% of these hypertensives were not previously known.⁵

Recognising the epidemiological transition from infectious to chronic non-communicable diseases, the Singapore government adopted a countrywide approach⁶ and since the 1990s, initiated various national health promotion⁷ and screening programmes.⁸

While treating hypertension may be one of the commonest clinical responsibilities of physicians, only 1 in 4 patients have their blood pressure adequately controlled.⁹ With Singapore slated to be one of the fastest ageing society, it is important to know the local prevalence of this cardiovascular risk factor and the current situation in terms of hypertension in community-dwelling elderly more than 65 years old and to explore some related issues.

Materials and Methods

The study was conducted over a period of 1 month from November to December 2002 in a suburban housing estate of Toa Payoh Central. Toa Payoh Central was chosen as the elderly population there was 11%,¹⁰ which is close to the current estimate of elderly population in Singapore. Furthermore, the ethnic distribution was 81% Chinese, 11% Malay and 7% Indian, closely resembling the current national ethnic distribution.

Out of an estimated 9000 households, 2300 households, stratified by housing type, were randomly selected by the Department of Statistics, Singapore. Of these, 346 households were excluded as these were either shophouses, from a private estate where the management declined participation or blocks designated to be demolished. Of the remaining 1954 households, there were no elderly in 1092 households (55.9%) and a one step replacement was done for these. During replacement, the same unit two floors below were chosen. When the unit happened to be on the second floor, the unit on the top floor was replaced. If the unit two floors below was already selected, another unit 4 floors below was chosen.

Of the 1954 households, 227 were not occupied and another 309 were not contactable despite making 3 visits at different times of the day. Some 151 households refused participation and 4 were not available, either on vacation, staying with another child or hospitalised during the period of study. A total of 171 households participated in the study.

A total of 921 households were replaced and, eventually, 60 households participated in the study. The overall household participation rate, considering households with elderly only, was

50.2%. The 231 (171 ± 60) households which participated in the study yielded 270 questionnaires, but 5 were excluded due to inaccurate or incomplete data. Finally, 265 elderly participated in the study.

A team of medical students were recruited, trained and sent to the homes of these elderly. A questionnaire was administered and it covered the medical and social history, such as a history of diabetes, stroke, ischaemic heart disease, smoking, alcohol consumption and living arrangements. The question on hypertension invited 4 responses: "hypertension on medication", "hypertension not on medication", "no history of hypertension" or "don't know". Where possible, the medications were recorded and correlated with the history given and the primary physician noted.

Blood pressure was taken twice after an oral interview, in the sitting position and using Omron T5 sphygmomanometer, and the average of both readings recorded.

The statistical package used was SPSS version 11.5.0. Chi-square test was used to evaluate significance of proportion.

Results

The prevalence of hypertension was 55.5% (n = 147) (Table I). The majority (63.3%) of those who had a history of hypertension were female. There was no dominance of any particular race in the hypertensive or non-hypertensive group. A history of hypertension was also positively associated with a history of diabetes and ischaemic heart disease. The mean age (± standard deviation) for both groups was similar: the hypertensive group was 72.6 ± 6.1 compared to 72.7 ± 6.1 in the non-hypertensive group.

Close to half (47.6%, n = 70) of those with hypertension were followed up at a polyclinic, 31.3% (n = 46) were followed up by private general practitioners and 17.0% (n = 25) followed by private or public hospitals.

TABLE I: SELECTED CHARACTERISTICS OF ELDERLY WITH AND WITHOUT HYPERTENSION

Variable	Hypertension (n = 147)		No hypertension (n = 104)		P
	n	(%)	n	(%)	
Male	54	(36.7)	55	(52.9)	0.011
Race					
Chinese	128	(87.1)	85	(81.7)	0.603
Malay	9	(6.1)	11	(10.6)	
Indian	8	(5.4)	6	(5.8)	
Others	2	(1.4)	2	(1.9)	
Alcohol drinker	13	(8.8)	15	(14.4)	0.158
Smoker	27	(18.4)	45	(43.3)	<0.001
History of stroke	15	(10.2)	7	(6.7)	0.330
History of diabetes	45	(30.6)	17	(16.3)	0.010
History of ischaemic heart disease	39	(26.5)	10	(9.6)	0.001

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The blood pressure readings of 246 out of 265 elderly, based on their history of hypertension, is presented in Table II. Using 140/90 mm Hg as a cut-off, 26.6% of elderly with hypertension had good control. The corresponding figure, using 160/90 mm Hg as a cut-off, was 54.0%.

TABLE II: BLOOD PRESSURE READING OF COMMUNITY-DWELLING ELDERLY

Variable	Blood pressure control level			Total
	<140/90 mmHg	≥140/90 to 160/90 mmHg	≥160/90 mmHg	
Hypertension/on medication	35	32	52	119
Hypertension/not on medication	2	6	12	20
No hypertension	33	26	34	93
Do not know	5	5	4	14
Total	75	69	102	246

There was no correlation between the level of control and the principal physician (Pearson's coefficient = 0.351). Neither was there a correlation between the level of control and race ($P = 0.409$), gender ($P = 0.090$), history of stroke ($P = 0.694$), ischaemic heart disease ($P = 0.068$) or diabetes ($P = 0.702$).

The potential rate of undiagnosed hypertension, using 160/90 mm Hg and 140/90 mm Hg as cut-offs, was 15.4% and 28.0%, respectively. There were slightly more males (51.7%) who had potentially undiagnosed hypertension but this was not statistically significant ($P = 0.584$). There was no relationship between those with potentially undiagnosed hypertension and race ($P = 0.42$).

Discussion

The finding that more than one in two of our community-dwelling elderly has hypertension highlights not just how common this cardiovascular risk factor is, but how seriously we should view the management of this condition. With a rapidly ageing society and a more sedentary and stressful lifestyle in the 40- to 64-year-old age group, this figure, both in terms of percentage and absolute numbers, can only be expected to increase. When hypertension is poorly managed, the resulting mortality and morbidity can be a huge medical and economic burden on our ageing society.

Our study showed that race and gender had little to do with hypertension or its level of control. This simplifies the picture, but complicates the strategy as no one particular group can be targetted for more focused action. This could also suggest a more fundamental underlying factor cutting across gender and race: our lifestyle in modern Singapore.

The lower rate (28%) of undiagnosed hypertension compared to the 53% recorded in 1998 by the National Health Survey, can probably be attributed to aggressive public education and community health screening undertaken by the relevant authorities. The next

challenge in hypertension management would appear to be showing the same reversal of proportions of those whose blood pressure is within target range. Close to 80% ($47.6\% \pm 31.3\%$) of those with hypertension were followed up by primary care physicians from both the public and private sectors and the emphasis should be on this group of doctors. The primary care physician often faces difficulties like compliance (often confounded by patients attributing unrelated symptoms to hypertensive medications), mindset and the delicate balance between cost and medications. This is particularly true as better control of hypertension often entails the use of more than one antihypertensive medication.⁹ While the practical difficulties of controlling hypertension should be acknowledged, a change in mindset on the part of the doctor that the elderly can be "allowed" to have higher blood pressure should be discouraged. Blood pressure monitoring at home should be encouraged to rule out white coat hypertension and postural fall in blood pressure actively looked for. Clinical judgement on a case-by-case basis is emphasised but, as far as possible, treatment of hypertension in the elderly can be more proactive and the patient encouraged to take ownership of his or her own health.

In conclusion, while national screening programmes and public education seem to be showing results with the apparent fall in the number of cases of undiagnosed hypertension, more proactive management and education are still necessary to improve the level of control and to bring about true disease prevention of this very important cardiovascular risk factor.

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Allogenic Stem Cells Transplantation in Rabbit Myocardial Infarction

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Introduction

It is generally accepted that cardiac myocytes lack the ability to regenerate after injury, which leads to permanent deficit in the number of functioning cardiomyocytes that contribute to the development and progression of heart failure. Cell therapy has emerged as a promising new strategy to supplement the deficient intrinsic repair mechanism of heart. Various types of cells, including fetal and adult cardiomyocytes and human skeletal myoblasts, have been used for cardiac transplantation. *In vitro* and *de milieu* dependent differentiation approaches are being pursued to generate cardiomyocytes from stem cells derived from different sources and have demonstrated great potential in treating heart failure. Stem cells are immature tissue precursor cells, capable of self-renewal and provision of replacement cells for many tissues. The present study involves transplantation of bone marrow-derived allogenic adult stem cells (BMCs) for cardiac repair. The results revealed that BMCs were able to survive in the infarcted myocardium in a rabbit model of myocardial infarction. The BMCs stimulated neovascularisation. An interesting feature of our findings is that some donor cells had transformed into endothelial cells and were seen as part of the blood vessel lumen.

Materials and Methods

Ten New Zealand white female rabbits (3~3.5 kg) were used in the study. The animals were divided into two groups: BMC-transplanted group (n = 6) and Dulbecco modified eagle's medium (DMEM) injected group (n = 4). All animals received humane care in accordance with the principles of research animals guidelines at the National University of Singapore.

The animals were premedicated with methohexitol (10 mg/kg im) and anaesthesia was maintained with isoflurane (2%) and 100% oxygen (2 L/min) using mechanical ventilation to maintain respiration. A left thoracotomy was performed on each animal and the heart was exposed. The first and second branches of the left circumflex coronary artery were ligated by suturing with prolene 4.0 and acute ischaemia was observed. The incision was closed and the animal was allowed to recover from anaesthesia. They were maintained on cephalosporin for 4 to 6 days.

Under general anaesthesia, 10 mL of bone marrow was aspirated using a bone marrow aspiration needle and cultured with 20% fetal bovine serum (FBS), 100 unit/mL penicillin G, 100 ug/mL streptomycin). The cells were incubated at 37°C in 5% carbon dioxide, and continually cultured for 4 weeks; the medium was changed every 2 to 3 days. As the haematopoietic stem cells do not attach themselves to the culture flask, they were removed with the culture medium changes.

To identify transplanted cells in the recipient myocardium, the cells were transfected with retrovirus encoding lac-z reporter gene for thrice. Briefly, 10 mL cell culture supernatant of FLYA4 cells containing 10⁵ viral particles/mL was filtered through a 0.22-um filter into each flask and incubated at 37°C in 5% CO₂. After 6 to 8

hours of transduction, another 10 mL of fresh DMEM was added and the cells were incubated for 24 hours. The transduction procedure was repeated over the next 3 days on 3 occasions. A day before transplantation, the BMCs were double labelled with BrdU. BrdU was diluted with DMEM at 1:600 and 15 mL of the diluted BrdU was added into the culture flasks and incubated for 24 hours at 37°C in 5% CO₂. The stromal cells were trypsinised using 0.05% trypsin, washed with PBS and 30 x 100⁶ cells were resuspended in 1 mL serum-free DMEM for transplantation.

At 2 to 3 weeks' post infarction, the animals were anaesthetised and the heart was exposed for the second time. BMCs suspended in 1 mL DMEM or DMEM without cells were injected into the centre and periphery of the infarct regions. The incision was sutured and the animals were maintained on cyclosporine at 5 mg/kg to prevent rejection of the donor cells. Antibiotics were administered for 5 to 7 days to prevent infection. At 1 week and 6 weeks post-transplantation, the animals were sacrificed, 3 each for the cell-transplanted group and 2 each for the DMEM-injected group. The hearts were harvested and processed for histological study.

The explanted heart tissue was embedded in freezing medium for cryosectioning. Sections of 6-8 um in thickness were cut and used for histochemical and immunohistochemical studies.

The tissue sections were fixed with 0.5% glutaraldehyde for 15 minutes at room temperature. After washing thrice with PBS for 10 minutes each, the slides were incubated with 5-bromo-3-indoyl-beta-D-galactosidase (x-gal) [40 mg/mL in N,N-dimethylformamide (DMF) in x-gal buffer containing 35 mM each of potassium ferricyanide, potassium ferrocyanide and 2 mM MgCl₂]. After 24 hours, the slides were rinsed in PBS and observed under microscope.

All sample slides were fixed in -20°C methanol for 10 minutes and rehydrated. The slides were blocked with 3% hydrogen peroxide thrice at room temperature and rinsed with PBS. After blocking with 1% BSA for 10 minutes, the slides were incubated with anti-BrdU primary antibodies, von Willebrand factor VIII or PBS in a moist chamber overnight at room temperature. The slides were incubated with secondary antibodies and after rinsing with PBS, the reaction was visualised with DAB substrate and photographed under microscope.

Results

BMCs that attached themselves to the base of the culture flasks proliferated within 3 to 4 days. The medium was changed every 2 to 3 days; the haematopoietic cells that did not attach to the flasks were washed away during the medium change (Fig. 1). The efficiency of lac-z transduction was 40% when stained with x-gal in vitro study. Fibrous tissue was observed in the infarcted region in both cell-transplanted heart and medium-injected heart. The transplanted cells were observed in transplanted heart tissue with positively stained BrdU and lac-z (Figs. 2, 3 and 5a). They survived in both infarct and peri-infarct regions. The medium-injected animal heart tissue stained negative for lac-z and BrdU expression (Fig. 5b). The donor cells

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were found following the tissue architecture. An interesting finding is that the donor BMCs were observed in the walls of the vasculature (Fig. 4). Immunostaining with von Willebrand factor VIII showed that the BMC-transplanted tissue showed more blood vessels compared to the DMEM-injected group (Figs. 6a and 6b).

Discussion

Myocardial transplants of cultured heart cells,¹⁻³ skeletal myoblasts,⁴⁻⁷ smooth muscle cells⁸ and bone marrow cells^{9,10} have been demonstrated to prevent heart failure after a myocardial injury. The advantages of bone marrow stromal cells include their easy availability and the possibility of differentiating them into cardiomyocytes that may form gap junctions and beat synchronously with the host myocardium.¹¹ In addition, they may differentiate into endothelial progenitor cells that may participate in neovascularisation after transplantation.¹² Tomita et al transplanted cultured bone marrow stem cells that were induced to differentiate into a myogenic phenotype with 5-azacytidine. This showed that the transplanted cells formed an island of cardiac-like tissue, induce angiogenesis and prevent thinning and dilatation of the infarct region. It also improved regional and global contractile functions.¹³ Orlic and associates transplanted bone marrow stem cells into an infarcted murine model. They showed a newly formed myocardium in the infarcted region. The newly developed tissue comprised proliferating myocytes and vascular structures.¹⁴ Kobayashi and coworkers also found that BMC implantation induced angiogenesis in a rat coronary artery ligation model.¹⁵ In our study, we showed that allogenic BMCs survived in the infarcted rabbit myocardium after transplantation. The donor cells survived both in infarct and normal myocardium. The lac-z and BrdU expression showed that they aligned following host myocardial architecture. vWFVIII staining demonstrated that the stromal cells secrete vascular endothelial growth factor; the transplantation of stromal cells accelerated neovascularisation.¹⁶ One limitation of our study is the small number of animals, which limited the evaluation of the cardiac function and statistics of our histological data. Further study is in progress to clarify the characteristics of transplanted cells.

Conclusion

Allogenic bone marrow stem cells can survive in both normal and infarcted regions of the heart. They can transform into endothelial cells and stimulate neovascularisation.

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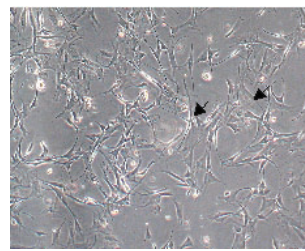


Fig. 1.

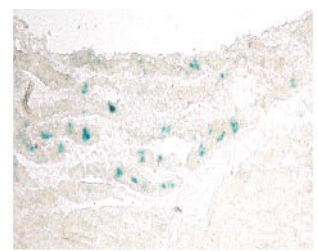


Fig. 2.

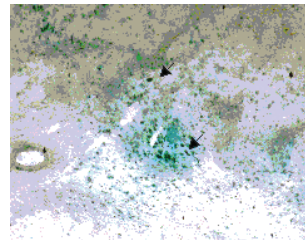


Fig. 3.

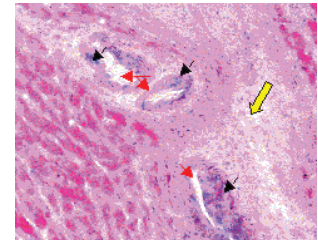


Fig. 4.

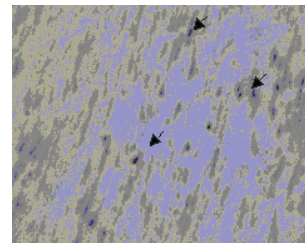


Fig. 5a.

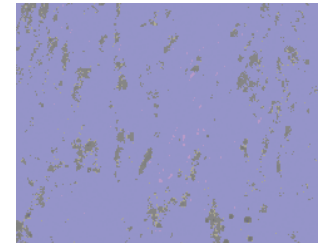


Fig. 5b.

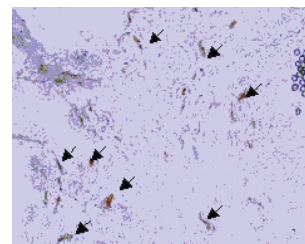


Fig. 6a.

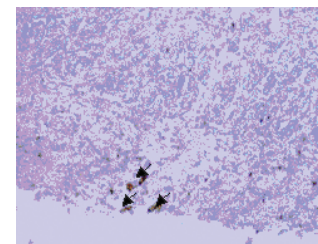


Fig. 6b.

Fig. 1. BMC in vitro culture at two weeks after bone marrow harvesting.

Fig. 2. BMC in the periphery of the infarcted region expressing lac-z reporter gene at 6 weeks after transplantation.

Fig. 3. Photomicrograph showing survival of lac-z expressing BMCs in the infarcted myocardium 1 week after transplantation.

Fig. 4. The transplanted BMCs transdifferentiated into endothelial cells and form the walls of the vasculature. The cells stained positive with x-gal formed the wall of blood vessel. Lac-z positive tissue counter stained with haematoxylin.

Fig. 5. The BMC-transplanted tissue stained for BrdU using anti-BrdU antibody.

(a) BMC of 6 weeks post-transplantation.

(b) DMEM-injected tissue stained negative for BrdU.

Fig. 6. The (a) BMC-transplanted tissue stained with factor VIII antibody for vascular density measurement. The BMC-transplanted tissue showed more neovascularisation. (b) DMEM-injected tissue.

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Special Poster Presentation

The Effect of Sialidase Inhibitors on Hyaluronan-mediated Escape from Apoptosis Induced by Anti-cancer Drugs in Gastric Cancer

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Introduction

Gastric adenocarcinoma is one of the leading causes of cancer death in Singapore. Surgery remains the main modality for cure, but this is no longer effective once metastasis occurs. Systemic chemotherapy by anti-cancer drugs is often useful, but the response is unpredictable. Moreover, the degree of differentiation of the gastric cancer also seems to affect the prognosis and pattern of metastasis. The reasons for these are unclear.

Metastasis is a multi-step cascade initiated when malignant cells break free from the primary tumour and enter the bloodstream, lymphatics or peritoneal cavity. Adhesion receptors on the surface of cancer cells play an important role in tumour cell migration, invasion and metastasis. A number of specific cell surface associated molecules that mediate cell-matrix and cell-cell interactions have been characterised. Cell-cell interactions are known to protect cells from apoptosis and may help to explain the chemoresistance of solid tumours.

CD44, an integral membrane glycoprotein expressed in many types of cells, is the principal transmembrane hyaluronate receptor and may be a determinant of metastatic and invasive behaviour. The sialylation process of CD44 plays a role in the conjugation of cells to hyaluronan (HA). HA is an important mediator of cancer apoptosis. The aim of our study is to observe the effect of HA on gastric cancer cell apoptosis induced by anti-cancer drugs and the influence of sialylation of cell surface adhesion molecules on this process.

Materials and Methods

Two gastric cancer cell lines (poorly differentiated MKN45 and moderately differentiated MKN74) were cultured and subjected to anti-cancer drug induced apoptosis and the HA inhibition test. 2×10^6 cells of exponential-phase culture were treated with etoposide (a topoisomerase inhibitor) and 5FU (a uracil analogue) at various concentrations for 12 hours at 37°C, in 5%CO₂. Apoptosis was measured by the propidium iodide stain method and the Hoechst 33258 stain method. The experiment was repeated with HA inhibition, with varying concentrations of HA from 5 to 25 µg/mL.

Next, CD44 expression in the cell lines was measured by indirect immunostaining under fluorescence microscope and flow cytometry. This was repeated after treatment with siastatin B between

10 and 100 µg/mL.

Next, the HA adhesion test was performed by coating flat-bottom plates with hyaluronate at 1 mg/mL and heat-denatured bovine serum albumin in phosphate-buffered saline at 10 mg/mL overnight at 4°C. 1×10^5 cells in a single-cell suspension were added and adhesion allowed to proceed for 1 hour at 4°C. The plates were inverted and unattached cells were aspirated. The plates were washed with phosphate buffered saline and viable cells were counted. The effect of two sialidase inhibitors, siastatin B and asialo-fetuin, on the HA adhesion test was studied at various concentrations.

Results

Apoptosis induced by the anti-cancer drugs was observed in a dose-dependant manner for both cell lines. Etoposide at 20 µg/mL and 5FU at 10 µg/mL induced apoptosis in 50% of culture cells. In the HA inhibition test, HA at concentrations between 5 and 25 µg/mL effectively prevented apoptosis induced by 20 µg/mL of etoposide in MKN45 cells ($P < 0.05$). This effect was not observed in MKN74 cells or in 5FU-induced apoptosis.

CD44 positive rate was 60% to 70% in MKN45 cells, but this could not be demonstrated in MKN74 cells. CD44 expression in MKN45 cells decreased after treatment with siastatin B between 10 and 100 µg/mL ($P < 0.05$), but not to asialo-fetuin.

In the HA adhesion test, sialidase inhibition by siastatin B and asialo-fetuin was found to decrease the gastric cell adhesion to HA in MKN45 cells ($P < 0.05$), but not in MKN74 cells.

Discussion

Apoptosis, or programmed cell death, is an orderly and genetically controlled process initiated either by ligand binding to cell surface receptors or by cell toxins via a pathway that targets the mitochondria. Most anti-cancer drugs act by activating an endogenous biochemical process for apoptosis. Malignant cells that develop defects in the genes that control apoptosis may render themselves resistant to chemotherapy.

CD44 is a family of transmembrane glycoprotein receptors for HA and this affinity can be reduced by alternative splicing and glycosylation. Cell surface CD44 overexpression can promote tumour cell survival in invaded tissues and its suppression can induce

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apoptosis of the invading tumour cells. There are only a few reports on the relationship between HA and apoptosis, and some of these are contradictory. Our findings suggest that adhesion to HA inhibited apoptosis induced by etoposide for the poorly differentiated MKN45 gastric cancer cells. This effect was not seen for 5FU. It has been reported that etoposide-induced apoptosis is inhibited by CD44 overexpression, possibly through a CD44-mediated signalling pathway. On the other hand, 5FU is known to decrease proliferation status and induce apoptosis, possibly via the Fas pathway. The different pathways of action for different anti-cancer drugs and their relationship with CD44 and HA may, therefore, account for our findings.

CD44 is acidic largely due to sialic acid. Sialic acids are a family of amino sugars that are commonly found as terminal oligosaccharide residues on glycoproteins and glycolipids. Glycosylation of CD44 itself can affect HA binding ability by negative regulation of its recognition of HA. Certain cells which had sialic acid removed by neuraminidase from their cell surface acquire the ability to bind HA. Analogues of sialic acid, such as neuraminidase inhibitors, can be potential anti-adhesion drugs. Our postulate that sialidase inhibitors can block the adhesion of gastric cancer cells to HA was verified in our HA inhibition test. This effect was achieved with siastatin B and asialo-fetuin for the poorly differentiated MKN45 cancer cells, but not in the moderately differentiated MKN74 cells. Siastatin B, a

multi-functional azasugar isolated from streptomyces culture, is an inhibitor of sialidase, while asialo-fetuin is a glycoprotein substrate for sialidase.

The differences in behaviour of the two cell lines in our experiment could be explained by their adhesive properties. The impact of our experiment was only limited to the low-adhesion, poorly differentiated MKN45 cell line, and not in the high-adhesion, moderately differentiated MKN74 cell line. Desialylation is higher in low-adhesion cell lines and may represent a potential target for therapy.

It was very interesting to find that siastatin B decreased the CD44 expression of MKN 45 cells in our experiment. It is generally thought that the variable glycosylations or splice variants determined its HA adhesion. CD44 is stable in the glycosylation process. Therefore, our findings suggest that HA adhesion might be important for CD44 splitting and, finally, CD44 expression.

Conclusion

Adhesion to HA may be an important mechanism for escape from apoptosis induced by anti-cancer drugs. This effect seems to be limited to poorly differentiated gastric cancer cells rather than moderately differentiated ones. Highly effective and specific sialidase inhibitors may modulate this effect. Our findings are preliminary but important, as it may lead to a novel and promising biologic therapy for increasing the chemosensitivity of anti-cancer drugs for gastric cancer.

Special Poster Presentation

Blood-Brain Barrier Disruption Following Traumatic Brain Injury: Roles of Tight Junction Proteins

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Introduction

The endothelial cells within the central nervous system (CNS) form a continuous wall joined by tight junctions with a high reflective coefficient and electrical resistance that prevents virtually all water-soluble substances from entering the CNS. This property, known as the blood-brain barrier (BBB), is pivotal in the maintenance of a stable and controlled microenvironment in the neuronal parenchyma by protecting against neurotoxic compounds and ionic concentration variations.¹ The tight junction consist of proteins, such as occludin,² claudin-1,^{3,4} ZO-1,⁵ ZO-2^{6,7} and ZO-3,⁸ which play important structural and accessory roles in the normal physiological function of the BBB.³

Intracranial hypertension is a major cause of secondary brain injury.⁹⁻¹¹ It may occur as a result of mass effect from a space-occupying lesion (haematoma or tumour) and/or brain swelling (vasogenic and cytotoxic),¹²⁻¹⁴ and contributes significantly to morbidity and mortality.

The contribution of BBB disruption following traumatic brain injury (TBI) to the development of intracranial hypertension is uncertain and is poorly understood at the molecular level. The proposed mechanisms of vasogenic oedema following TBI and intracranial hypertension include opening of tight junctions, enhanced vesicular transport and damage of endothelial membranes. In this study, we attempted to study the roles of tight junction protein (occludin, claudin-1, ZO-1, ZO-2 and ZO-3) as a result of TBI.

Materials and Methods

Fluid Percussion Injury

35 male Sprague-Dawley rats were randomly assigned to sham-operated group and lateral fluid percussion injured group. They were further divided into 3 subgroups for different experimental procedures: 15 for brain water content, 10 for immunohistochemistry analysis of tight junction proteins and 10 for horseradish peroxidase (HRP) staining procedures.

The lateral fluid percussion injury was induced in the rats based on

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the protocol described by McIntosh et al.¹⁵ The rats were anaesthetised with a concoction of xylazine (10 mg/kg) and ketamine (150 mg/kg) intraperitoneally and allowed breathing spontaneously. A 5-mm craniotomy was made over the left parietal cortex midway between the lambda and bregma sutures. A Leur-Loc was tightly fitted and cemented to the craniotomy with dental acrylic. Injury was induced by trephination, with the impact of the rapid fluid bolus that strikes the intact dural surface producing a focal injury to the brain.¹⁶ The impact of the fluid bolus was measured in atmospheres.

Brain Water Content

The animals were sacrificed after 2 hours post-injury for both the study and sham-operated groups. The brains were removed immediately and weighed before being dehydrated at 90 degree Celsius for 72 hours.¹⁷ The brain samples were weighed again and the brain water content is estimated using the formula depicted below:

$$1 - (\text{weight after drying} / \text{weight before drying}) * 100\%$$

Horseradish Peroxidase

The animals were administered with 80 mg/kg (dissolved in 3 mL) of HRP Type VI (Sigma Chemical Co., St Louis, Mo) 5 minutes post-injury for the lateral fluid percussion injured group and post-surgery for the sham-operated group via the femoral artery over a 5-minute period. They were sacrificed 120 minutes post-administration of the HRP. The animals were perfused with 4% paraformaldehyde. The brains were removed and immersed in 4% paraformaldehyde for 24 hours and then transferred to 20% sucrose for another 24 hours at 4 degree Celsius. Sections of 40 mm mounted on poly-L-Lysine treated slides were then washed thrice with PBS. The slides were immersed in 3% hydrogen peroxide for 10 minutes and washed again thrice with PBS. The slides were stained with DAB for 10 minutes and washed thrice with deionised water before being mounted with coverslip.

Immunohistochemistry and Analysis

The brain samples were prepared in the same manner as above without the administration of HRP. Immunoreactive ZO-1, ZO-2, ZO-3, claudin-1 and occludin were detected by the labelled streptavidin-biotin method. Sections of 10 mm mounted on poly-L-Lysine-treated slides were extensively rinsed in PBS and endogenous peroxidases were quenched in 3% hydrogen peroxide for 5 minutes. After washing in PBS, slides were incubated with blocking reagent for 30 minutes (Dako LSAB Kit, Dako Carpinteria CA). Incubation with primary antibody was undertaken at 4°C overnight with a 1:50 dilution of rabbit anti-ZO-1 polyclonal antibody, rabbit anti-ZO-2 polyclonal antibody, rabbit anti-claudin-1 polyclonal antibody, rabbit anti-occludin polyclonal antibody (Zymed Laboratories Inc., San Francisco, CA, USA) and mouse anti-ZO-3 monoclonal antibody (Chemicon International, Temecula, CA, USA). Following three rinses with PBS, slides were incubated with linking antibody (Dako) for 10 minutes before washing with PBS, and followed by 10 minutes with streptavidin-HRP diluted as recommended by the manufacturer and incubated for 8 minutes with the chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB; Dako). After incubation, samples were rinsed thrice with deionised water. Samples were counterstained with 0.5% methyl green for 1 minute. Slides were dehydrated and mounted.

Results

All animals were monitored haemodynamically post-injury and none suffered hypotension and/ or hypoxia following injury. Brain oedema was evident two hours after trauma; the brain's water content in the injury group was higher than that in the sham-operated group ($83.97 \pm 0.21\%$, $n = 10$ versus $82.11 \pm 0.6\%$, $n = 5$) ($p < 0.05$)

(Fig. 1).

Immunohistochemically, all the tight junction proteins studied (ZO-1, ZO-2, ZO-3, claudin-1 and occludin) showed punctuated expression in the paracellular pathway between endothelial cells, evidenced by the patchy chromogen staining compared with the tight junctions in the healthy rat brain sections of the sham-operated group (Fig. 2). This staining loss was seen not only at the penumbral region of the injury created, but also further afield in the ipsilateral hippocampus (Fig. 2).

Light microscopy also demonstrated extravasation of HRP into the interstitial space 2 hours following lateral fluid percussion injury. At lower magnification, we observed localised HRP extravasation in the cortex and hippocampal region in the ipsilateral hemisphere of injured rat brain. At higher magnification, the HRP is phagocytosed by the microglial cells (Fig. 3).

Discussion

Increased intracranial pressure (ICP) is a frequent accompaniment following TBI and intractable intracranial hypertension has been identified as a significant cause of mortality in the neurocritical care setting. The primary injury and subsequent secondary insults contribute to neuronal cell demise via multiple pathways, such as excitotoxicity, apoptosis and lipid peroxidation. One of the presumed injurious events that occur is a breakdown of the BBB with extravasation of fluid into the extracellular compartment, with its attendant deleterious effects on cell membrane function and metabolism.^{18,19} Nevertheless, to date there are no reports on the characterisation of the biological derangement of the BBB with regards to the effect of TBI on the protein structural integrity that maintains the BBB.

The structural basis of the BBB has been the subject of intense study and its elaborate network of complex tight junctions has been investigated morphologically by freeze-fracturing and ultrathin sectioning and physiologically by measurements of paracellular permeability and transepithelial/transendothelial electrical resistance.^{20,21} The molecular organisation of the BBB tight junctions has been elucidated and consists of occludin,^{2,22} claudin-1,³ ZO-1,⁵ ZO-2,⁷ and ZO-3.⁸ While occludin is a transmembrane protein of approximately 65 kDa, ZO-1 (220 kDa), ZO-2 and ZO-3 are submembrane proteins and claudin-1 is a major cell adhesion molecule of the tight junction.

In our study, the BBB appeared from a morphological perspective to be altered by TBI significantly at 2 hours post-injury. We employed 2 methods to prove that punctuated expression of the tight junction proteins was the cause of BBB disruption, namely, brain water content and HRP assessment. At 2 hours, the ipsilateral hippocampus showed patchy loss of occludin, claudin-1, ZO-1, ZO-2 to ZO-3 protein expression. This loss of immuno-expression correlated with the increase in brain water content and HRP extravasation, suggesting that there was developing vasogenic oedema from tight junction disruption. To date this is the first publication that documents the patchy disruption of the BBB following TBI. What is important was, in addition to the localised areas of BBB disruption at the site of the contusion was the evidence further afield (hippocampus). The interrupted protein expression may be caused by endothelial cell injury from the effects of mediators, such as bradykinin, arachidonic acid, histamine, free radicals and polyamines.^{23,24}

Modulation of protein expression has been employed as a therapeutic strategy in a wide variety of degenerative and psychiatric disorders to good effect. Tight junction proteins, in the same manner, bear promise as a target for pharmacologic intervention. Also, this new finding has important implications as the understanding of the

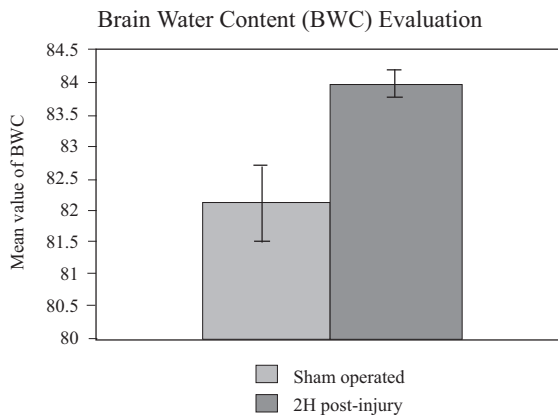


Fig. 1. Brain water content (BWC) were evaluated using the wet/dry weight technique. Results show that brain odema was evident 2 hours after trauma, the brain's water content in the injury group ($83.97 \pm 0.21\%$, $n = 10$) was higher than that in the sham-operated group ($82.11 \pm 0.60\%$, $n = 5$) ($P < 0.05$).

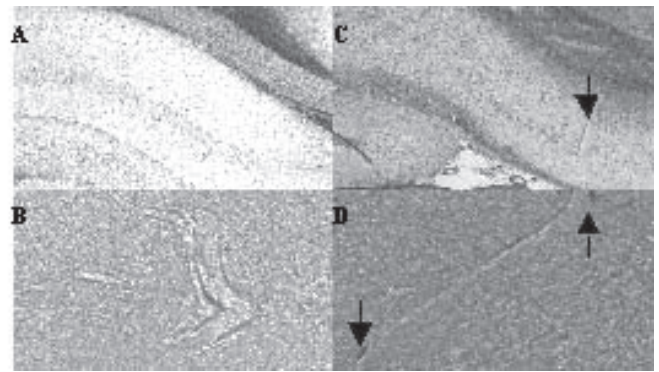


Fig. 3. Light microscopy showing extravasation of horseradish peroxidase (HRP) into the extracellular compartment 2 hours following lateral fluid percussion injury. HRP were infused 5 minutes post-surgery (for the sham-operated) and post-injury (for the injury group) via the femoral artery, and the rats were sacrificed 2 hours later. A-B show lower (50X) and higher (400X) magnification of the healthy rat brain section from the sham-operated group with no evidence of HRP extravasation. C — At lower magnification (50X), observations of localised HRP extravasation were found in the cortex and hippocampal region in the ipsilateral hemisphere of injured rat brain. D — At higher magnification of 400X, it can be seen that the HRP were phagocytosed by the microglial cells. The HRP were stained with DAB chromogen.

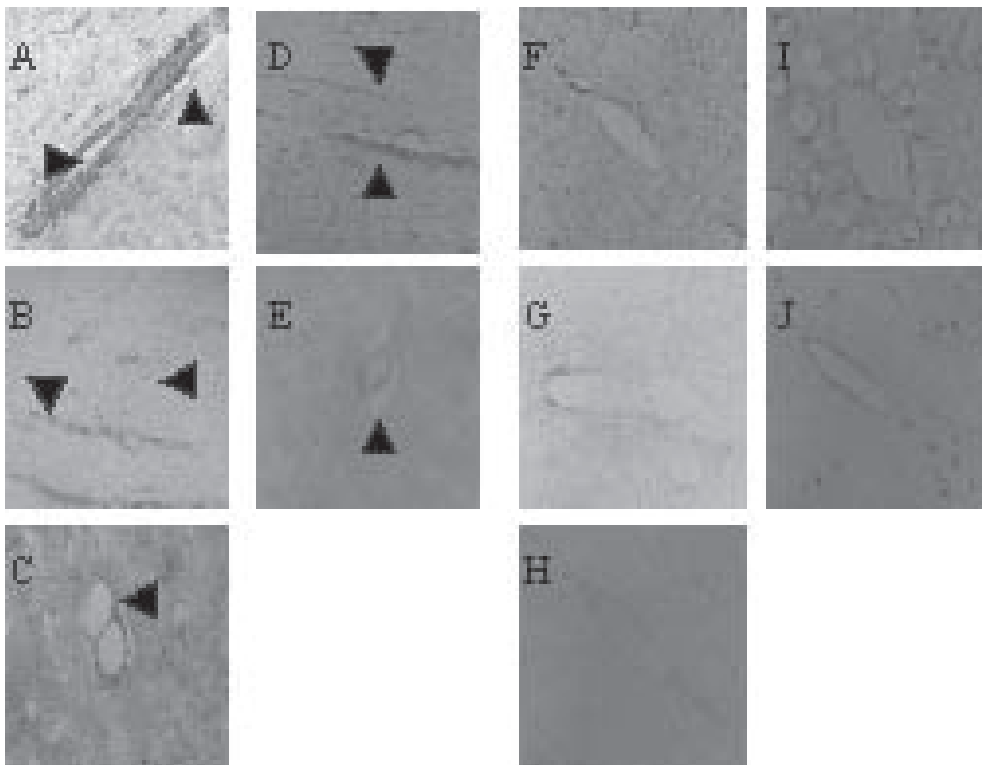


Fig. 2. Immunohistochemistry analysis of changes in the tight junction proteins morphology, the structural basis of the BBB, after lateral fluid percussion injury in rats. A-E (arrows) show the clear outline of tight junction proteins drawing individual endothelial cell's boundary with immunoreactive ZO-1, ZO-2, ZO-3, Claudin-1 and Occludin antibodies respectively. The lining of the endothelial cells of the blood vessel wall in the hippocampal region of the sham-operated rat brain were stained with DAB chromogen and observed at 400X magnification. F-J show the punctuated pattern of the outline that encircle each individual endothelial cells in the brain sections of rats 2 hours following lateral fluid percussion injury. Note especially the tight junction proteins in the paracellular pathway between 2 individual endothelial cells are compromised, evident with the absence of chromogen staining which were normally found in the healthy rat brain sections of the sham-operated group.

basic molecular pathology of the BBB disruption may lead to new avenues of delivering drugs into the CNS. For instance, vascular endothelial growth factor (VEGF) has been shown to reduce occludin expression. It is conceivable that blocking VEGF expression, which presumably is produced as a result of hypoxia, can lead to the preservation of the BBB in pathological states.²⁵

Conclusion

Following TBI, there is a punctuated expression of tight junction proteins that constitute the BBB. This appears to be associated with the development of vasogenic oedema that can contribute to ICP. Understanding the molecular mechanism of BBB breakdown may facilitate novel therapeutic intervention to preserve this barrier and prevent cerebral oedema.

Acknowledgement

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Predictors of Success in Smoking Cessation Among Hospitalised Patients

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Introduction

The aim of this study was to determine the clinical factors affecting smoking abstinence in hospitalised patients receiving a smoking cessation intervention that is considered adequately intensive for effectiveness based on current evidence.¹⁻³

Materials and Methods

This prospective study was conducted in a university-affiliated urban hospital. The recruitment sites for the study were 4 general wards of the hospital. These 4 wards admitted patients from the cardiology and respiratory medicine departments. The participants recruited were current smokers, 19 years of age or older, of stable condition to receive the intervention, had no gross memory impairment, were literate and had access to a telephone for follow-up intervention.

A dedicated smoking counsellor was employed to contact patients within 3 days of admission, to ask if they would be willing to talk about smoking. Those who agreed received a 30-minute behavioural counselling session provided at the bedside. Beginning with an assessment of the patients' intentions concerning smoking cessation,^{4,5} the counselling was tailored accordingly. The intervention was based on 5 principles: (1) smoking cessation is a process, (2) individuals choose to quit smoking; they cannot be forced, (3) smoking interventions need to be individualised and stage-matched, (4) people perceive that they have the skills and ability to quit smoking if they are to be successful (smoking cessation self-efficacy), and (5) cessation efforts must be reinforced with follow-up.

The following were components of the intervention: (1) a bedside counselling session, (2) written self-help material, (3) a prompt in the medical records for physicians and (4) counselling via telephone after discharge. The counsellor reviewed the dangers of smoking and the benefits of quitting; assessed participants' knowledge and beliefs about, and potential barriers to smoking cessation; and provided counter-arguments to belief barriers. Behavioural self-management techniques to counter known relapse triggers, such as stress, the presence of other smokers, alcohol use and depression, were also discussed. Subjects were encouraged to set a firm quit date, preferably that same day; the focus was on making a decision to maintain abstinence in the hospital and after discharge. At the close of the session, the patients were given the booklet "Quit Kit",⁶ printed in whichever language (English, Chinese, Malay or Tamil) the patients preferred, and were asked to review it. A sticker was placed on each counselled patient's hospital notes to remind the staff and physician to advise smoking cessation at future contacts.

Patients who met the criteria for nicotine dependence based on the use of the modified Fagerstrom Tolerance Questionnaire,⁷ or who were noted to have severe withdrawal symptoms during hospitalisation, were offered nicotine replacement therapy. The smoking counsellor obtained prescriptions from primary physicians and instructed patients on the appropriate use of either the nicotine patch or inhaler. Verbal instructions on the use of these agents,

including a schedule for decreasing use after 8 to 12 weeks were provided.

Data collection procedures were like medical record extraction from the subject's medical records for current medical information, and for medical history and risk factor data through the use of a standardised data collection form. The baseline information collected include: (1) sociodemographic factors; (2) medical history; (3) smoking history (pack-years, usual and recent cigarette use, and number of prior quit attempts); (4) nicotine withdrawal symptoms; (5) readiness (i.e., intention) to stop smoking; and (6) patient beliefs about the relationship between smoking and their health. After discharge, participants received follow-up telephone counselling calls from the same smoking counsellor. These calls were made at fortnightly intervals during the first post-discharge month and then monthly intervals for the following 2 months. These telephone counselling and follow-up sessions continued the skills training initiated during the face-to-face counselling session, and each telephone counselling call lasted less than 30 minutes. Participants who had resumed smoking were encouraged to set new quit dates.

Participants were defined as being continuously abstinent if they reported that they had not smoked at all in the past week. They also had to have an expired carbon monoxide (CO) reading <10 ppm. Data at the 2-month follow-up were collected at the time of routine hospital visit after discharge. Patients unavailable for follow-up were excluded. Patients with self-confessed abstinence with refusal of expired CO testing during follow-up were considered to be smokers.

Univariate and multivariate analyses were performed to identify baseline characteristics that were associated with smoking cessation after hospital discharge. In the univariate analysis, the independent student t-test was used to compare continuous variables, whereas the chi-square test was used for categorical variables. Significant factors associated with cessation in the univariate analysis (i.e., $p < 0.05$) were included sequentially in the multivariate logistic regression model,⁸ starting from the most significant variable. The dependent variable was expired CO-validated one-week abstinence at 1 month after discharge. Data were analysed using Stata version 6.0.

Results

Of the 268 patients included in the study over a 6-month recruitment period, 227 (84.7%) were men; the mean age was 56 years. The baseline characteristics of the patients are shown in Table I. The number of patients with cardiology and respiratory diagnoses were 137 (51.1%) and 123 (45.9%), respectively. The baseline smoking history of the patients are as follows: mean ($\pm$ SD) age started smoking was 18.7 ± 7.5 years; mean duration of smoking was 37.4 ± 15.3 years; median number of cigarettes was 15 (range, 1 to 80). All patients had considered that smoking worsened their condition.

All patients survived to hospital discharge. Two patients died before the 2-month follow-up and were excluded from the analysis. At 2 months after hospital discharge, 108 patients were continuously non-smoking and 106 were still smoking. Fifty-two patients had

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TABLE I: BASELINE CHARACTERISTICS OF SUBJECTS

Variable	No. of subjects (n = 268)
Sex	
Female	41 (15.3)
Male	227 (84.7)
Race	
Chinese	186 (69.4)
Malay	39 (14.6)
Indian	36 (13.4)
Others	7 (2.6)
Highest education level	
Nil	54 (20.2)
Primary	112 (41.8)
Secondary	89 (33.2)
Diploma/Degree	13 (4.9)
Marital status	
Single	33 (12.3)
Married	209 (78.0)
Divorced/Separated/Widowed	26 (9.7)

TABLE II: UNIVARIATE ANALYSIS OF DEMOGRAPHIC AND CLINICAL FACTORS ASSOCIATED WITH SMOKING CESSATION

Variable	Still smoking (n = 106)	Quit smoking (n = 108)	p value ¹
Mean age (SD) [y]	55.8 (15.2)	56.7 (15.1)	0.675
Sex			
Female	17 (16.0%)	16 (14.8%)	0.804
Male	89 (84.0%)	92 (85.2%)	
Race			
Chinese	73 (68.9%)	77 (71.3%)	0.562
Malay	15 (14.2%)	17 (15.7%)	
Indian	14 (13.2%)	13 (12.0%)	
Others	4 (3.8%)	1 (0.9%)	
Marital status			
Single	16 (15.1%)	10 (9.3%)	0.212
Married	79 (74.5%)	91 (84.3%)	
Divorced/Separated/Widowed	11 (10.4%)	7 (6.5%)	
Highest education level			
Nil	20 (18.9%)	19 (17.6%)	0.547
Primary	42 (39.6%)	51 (47.2%)	
Secondary	39 (36.8%)	31 (28.7%)	
Diploma/Degree	5 (4.7%)	7 (6.5%)	
Requirement of intensive care			
No	86 (81.1%)	77 (71.3%)	0.091
Yes	20 (18.9%)	31 (28.7%)	
Hospitalisation			
Initial	10 (9.4%)	2 (1.9%)	0.016
Recurrent	96 (90.6%)	106 (98.2%)	
Principal diagnoses			
Cardiology	54 (50.9%)	65 (60.2%)	0.174
Respiratory	52 (49.1%)	43 (39.8%)	
Mean duration of hospitalisation (SD) [days]	4.8 (3.4)	5.3 (4.0)	0.348

¹ Independent t-tests were used to compare means of continuous variables, whereas the chi-square test was used for categorical variables.

defaulted on their follow-up and were not contactable. These 52 patients were also not considered in the analysis. Hence, the smoking cessation rate at 2 months after discharge was 50.5%.

Univariate analysis of factors that may affect smoking cessation are shown in Tables II and III. There is no significant difference in demographic and clinical factors between quitters and non-quitters, except a significantly greater proportion of patients admitted for the first time for their medical condition quit smoking 2 months after hospital discharge ($p = 0.016$). For factors related to smoking, successful quitters had an older age of onset of smoking ($p = 0.005$), were smoking fewer cigarettes per day ($p = 0.016$) and had made more numbers of attempt to quit ($p < 0.001$). There were also significantly greater proportions of patients in higher stages of readiness to stop smoking ($p < 0.001$), preferring to quit “cold

TABLE III: UNIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH SMOKING CESSATION

Variable	Still smoking (n = 106)	Quit smoking (n = 108)	p value ¹
Mean age started smoking (SD) [y]	17.4 (6.7)	20.3 (8.0)	0.005
Mean years of smoking (SD)	37.9 (15.6)	36.4 (15.2)	0.086
Median number of cigarettes smoked per day at baseline (range)	17 (2-60)	10 (1-40)	0.016 ^f
Median number of quit attempts (range)	0 (0-10)	1 (0-3)	<0.001 ^f
Stage of readiness to quit			
Action	0 (0.0%)	1 (0.9%)	<0.001
Contemplation	71 (67.0%)	100 (92.6%)	
Pre-contemplation	35 (33.0%)	7 (6.5%)	
Preferred method of quitting			
Sudden cessation	78 (73.6%)	97 (90.1%)	0.002
Reduction	28 (26.4%)	11 (9.9%)	
Nicotine dependence score			
Low	49 (46.2%)	76 (70.4%)	<0.001
High	57 (53.8%)	32 (29.6%)	
Smoking history in family			
No	69 (65.1%)	73 (67.6%)	0.699
Yes	37 (34.9%)	35 (32.4%)	

^f Number of cigarettes smoked and number of quit attempts were analysed on the log scale, due to skewness of distribution.

¹ Independent t-tests were used to compare means of continuous variables, whereas the chi-square test was used for categorical variables.

TABLE IV: MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH SMOKING CESSATION USING LOGISTIC REGRESSION METHOD

Covariate	OR	95% for OR	p value ¹
Preferred method of quitting			
Reducing	1		
Nicotine replacement therapy	0.87	(0.27-2.78)	0.811
Cold turkey	8.33	(1.72-50.00)	0.009
Nicotine dependence score			
High	1		
Low	2.78	(1.37-5.56)	0.004
Cases/first-timers			
No	1		
Yes	8.35	(1.64-42.63)	0.011

¹ p values from Wald test.

turkey” ($p = 0.002$) and with low nicotine dependence score (Fagerstrom test for nicotine dependence ≥ 5 points; $p < 0.001$) among successful quitters. Multivariate analysis showed that a low nicotine dependence score (odds ratio 2.78, 95% CI: 1.37-5.56, $p = 0.004$), determination to quit by the “cold-turkey” method compared to reducing (odds ratio 8.33, 95% CI: 1.72-50.00, $p = 0.009$) and initial hospitalisation (odds ratio 8.35, 95% CI: 1.64-42.63, $p = 0.011$) were the main independent predictors of positive outcome (Table IV).

Discussion

In a systematic review on interventions for smoking cessation in hospitalised patients,¹ the analyses by diagnosis suggested that there was an increase in cessation rate in patients with cardiovascular diagnosis given an intervention, while the effectiveness of interventions in patients with a respiratory diagnosis was unclear. Our results did not show any significant increase in cessation rate in patients with cardiovascular diagnosis and respiratory diagnosis. Patients with different clinical diagnoses do not appear to respond in different ways to the intervention. Although different diagnoses may provide greater or less motivation to make a quit attempt, other factors are likely to be more determining.

Another critical factor of the hospitalisation that may influence outcome was whether the hospitalisation episode was the first for the patient’s medical problem. To our knowledge, this is the first study

that has shown that, among hospitalised smokers, quit rates for smoking cessation intervention provided for patients during the initial hospitalisation are higher than those given to patients with repeated admissions. It may be that patients are more receptive to the intervention during and following their first hospitalisation for the medical illness compared to repeated hospitalisation for the same illness. If this is so, it may have significance in designing future hospital-based cessation programmes targeting the most receptive patients in order to achieve the best possible outcomes.

Biological factors, such as experiencing nicotine withdrawal symptoms and the level of nicotine dependence, affect cessation rates in the general population and are also likely to play a substantial role in successful cessation among hospitalised patients. In the present study, a low nicotine dependence score was an independent predictor of smoking cessation. However, the introduction of nicotine replacement therapy (NRT) does not seem to have significantly affected quit rates among hospitalised patients in general. Taylor et al¹⁰ permitted the use of nicotine gum or patch for participants who met the criteria for nicotine dependence or who had severe withdrawal symptoms. There was no significant difference between the quit rate of the NRT group and that of the group without NRT.

Conclusion

Among a cohort of patients hospitalised with cardiac or respiratory

diagnoses and receiving smoking cessation intervention, initial hospitalisation, low dependence on nicotine and motivation to quit by sudden cessation are the main independent predictors of positive outcome. Patients with different clinical diagnoses do not appear to respond in different ways to the intervention.

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Special Poster Presentation

Caregiver Stress in Activities of Daily Living-dependent Elderly Aged More Than 65 Years

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Introduction

The Asia-Pacific region has more than half of the world's population. Over the next 25 years, this region would contribute significantly to a worldwide doubling of people who are more than 65 years of age.¹ People would have a longer lifespan, a result of good socio-economic progress and improvement in modern medicine. With longer lifespan, the proportion of frail elderly with disabilities as a result of birth, accidents, chronic illnesses and even ageing would only increase. Faced with the challenges of providing sufficient and adequate care for the elderly, many governments have begun the process of devolving health-related care from institutions onto the communities and homes.²⁻⁴ Singapore is in a similar situation, with an expected 19% of her population, or 796,000 people, aged >65 years old in 2003. About 10% of the elderly needs assistance in basic activities of daily living (ADL).⁵

The family has been the traditional caregiver for the frail elderly.^{6,7} This form of care is now under threat as family size decreases and more women, traditionally the caregivers, entered the workforce.

Caregiver provides direct care, like bathing, and indirect care, like

running errands. Psychodynamic issues are involved in this type of relationship that may precipitate feelings of resentment and depression. Stress on the caregiver will only increase as the care recipient grows older with functional decline, dwindling financial resources and less family members to share the care burden.⁷

Caregiver stress could be expressed in somatic complaints,⁸ hypertension, poorer immune functions, coronary heart disease,⁹ physical and financial burdens.⁷ The caregiver may be able to cope, as in the "adaptation" hypothesis, or suffer, as in the "wear and tear" model,^{10,11} with dire consequences for the frail elderly: neglect, abuse and, finally, institutionalisation.

In this study, we attempt to assess caregivers' issues associated with caring for community-dwelling ADL-dependent elderly.

Materials and Methods

This study is part of a larger community-based study on the health of elderly in Singapore. This study was conducted in November and December in 2002 in Toa Payoh Central. This area has an estimated 9000 households with about 11% elderly population, which is close

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to the national population estimate. Of the 2300 households randomly selected by the Department of Statistics, Singapore, a final 265 elderly participated in that study after taking into consideration households that declined, uninhabited, uncontactable and appropriate one-step replacement for elderly-absent households. Of the 54 ADL-dependent (based on Barthel Index) elderly with caregivers, only 76% (n = 41) of caregivers participated in the caregiver section.

The Zarit Burden Interview¹² was used to assess the impact of the elderly's disabilities on the caregiver's physical, emotional, social and financial well-being. An estimate of the degree of burden could be made on the total score (0-20: little or no burden; 21-40: mild to moderate burden; 41-60: moderate to severe burden and 61-88: severe burden). Issues of utilisation of support services (day care centres, day rehabilitation centres, home medical or nursing services and home help services) and finances were explored. Statistical analysis was performed using SPSS version 11.0.

Results

The elderly were generally older than the general study population: 75.2 years versus 71.7 years for male and 77.4 years versus 73.4 years for female. More females (n = 31) were present and this could be attributed to their longer life expectancy. The average Barthel Index was 75.5 for male and 70.8 for female. Most stayed with their children (82.9%, n = 34), while 12.2% (n = 5) stayed with their spouse and 2.4% (n = 1) stayed alone. Majority of the caregivers were children (51.2%, n = 21); spouse and maids, 19.5% (n = 8) each. The mean Zarit Burden Interview score was 17.9 ± 14.1 . Majority [53.7% (n = 22)] of caregivers expressed little or no burden; 39% (n = 16) had mild to moderate burden; 7.3% (n = 3) had moderate to severe burden; and none had severe burden. Up to 87.5% (n = 35) of caregivers felt sometimes that they should do more for the elderly and, similarly, up to 82.9% (n = 34) felt sometimes that they could do a better job in caring. Only 4.9% (n = 2) of caregivers utilised community support services, while up to 32.5% (n = 13) were not aware of their existence. Between 65% and 78% (n = 26 to 32) were aware, but not using the services. About 82.1% (n = 32) of caregivers spent up to \$600 monthly on nursing the elderly; 82.1% (n = 32) of the households surveyed had <\$3000 in total monthly income. Children were the main financial source (57.5%, n = 23), followed by self (15%, n = 6) and spouse (10%, n = 4). Insurance was notably absent as a source of income.

Discussion

In this study, the majority of caregivers [92.7% (n = 38)] were not overstretched in their roles as expressed in the Zarit Burden Interview score. Possible contributing factors may be due to:

- The emotional, social and physical well being of the caregivers were not threatened significantly. As the current caregivers are the baby boomers from the 1960s' cohort, the burden of parental care could be shared among the siblings.
- The elderly in this study were not severely ADL-dependent, and, hence did not pose a strain on the type of care required.
- Attitude of caregivers – not many had expressed a need to do more for the elderly, nor the necessity to do a better job in caring for the elderly.

The caregivers may need to have a mindset change with regards to care for the elderly. The attitude of ageism need to be corrected. Medical and nursing needs may change with time and may become more sophisticated; similarly, expectations of better educated elderly may increased. It would be appropriate to educate and train the caregivers to recognise such changes in the elderly and to respond appropriately. With appropriate input, the functional decline of the elderly could be stabilised or even improved upon.¹³ On the perception

of higher stress burden of caregivers are factors such as greater involvement in provision of care, especially of elderly with higher level of disabilities, increased duration of care, lack of personal and social resources, lack of family support and lower family income.¹⁴⁻¹⁶

Of particular concern would be a group of elderly who stayed with their spouses only. Spousal care may become more prominent as more elderly opted to stay away from their children in years to come.¹⁷ The spouses themselves are particularly at risk of stress, as they too grow old, with the attendant functional decline and less family support as the family unit becomes smaller.⁷

Paid help, in the form of maids, may become a prominent source of care for the elderly. They are usually untrained and need to be equipped with skills in order to fulfil the caregiver role. They would need to be orientated to the peculiar cultural aspects of the local population in order to better relate and play a more effective role in caring for the elderly. Formal caregivers would, in time, play a more prominent role as the availability of informal caregivers (usually family members) declined.¹⁸

Financial concerns were raised in 12% (n = 5) of caregivers who answered the Burden Interview. This could be due to the fact that an estimate of 20% (\$600/\$3000) of the household income may be spent on caring for the elderly at home. Also, 17.5% (n = 7) of caregivers had expressed concerns that the financial source may not be regular. The causes for the latter statement may be related to job security, especially in the current economic down cycle of the late 1990s and early 2000s, increasing medical expenses and decreased contribution from a decreasing family size. Currently, the efforts of the caregivers are not fully recognised financially and they may have to give up their jobs.

More studies would be needed to evaluate the causes for poor utilisation of community support resources. These could be attributed to the cost of utilisation, proximity, mobilisation, transportation, time constraints, attitudes of caregivers and care recipients, and type and appropriateness of facilities available. More effective communication to the caregivers, care recipients and even primary care personnel may be needed.

There are positive effects on caregivers with the appropriate matching of community support resources.¹⁴ These include respite care, better home care management, network with other potential available resources, professional help advice, reduce family breakdown and chance of partial employment. The main objective of such resources would be to reduce caregiver burden through assisting and supplementing the caregiver in his/her role.

The WHO Study Group on home-based long-term care⁶ has summarised that home care of the sick is not just fulfilling a physical need, but a social responsibility and an element in development of the individual and society. While allowing the frail elderly to reap the advantages of home care, the needs of caregivers should not be neglected. The caregiver should receive societal support in order to effectively perform his/her role so that the frail elderly may receive the best benefits. If the caregiver is not well managed, the latter may become another victim of poor health who needs help himself/herself.

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Special Poster Presentation

Interleukin-4 Receptor α Subunit (IL-4RA) Gene Polymorphisms and Singapore Childhood Minimal Change Disease

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Introduction

Minimal change nephrotic syndrome (MCNS) is a glomerular disease that is characterised by heavy proteinuria and a relapsing/remitting course, without histological evidence of classical inflammatory immune-mediated injury.¹ It is the most common cause of nephrotic syndrome in Singapore children. Although its pathogenesis remains to be elucidated, there is strong evidence that it is a primary immune disease associated with immunoregulatory imbalance between Th1 and Th2 cytokines.²

We have demonstrated that gene expression of interleukin-13 (IL-13) was upregulated in both CD4+ and CD8+ T-cells in children with steroid-responsive nephrotic syndrome in relapse, compared to remission, and this was associated with increased intra-cytoplasmic IL-13 production by CD3+ cells,³ and elevated serum total IgE levels during relapses. Moreover, the elevated serum total IgE levels found in patients in nephrotic relapse correlated significantly with the increase in CD3+ IL-13 producing-cells. Other studies have also indicated an important functional role of IL-13 in the pathogenesis of MCNS. Recently, IL-13 has also come to be appreciated as a critical mediator both functionally and genetically in some immune disorders such as allergic disease.

Whether the genetic polymorphisms of IL-13 or its receptors are associated with nephrotic syndrome has thus triggered much attention. We have found that the 3 single nucleotide polymorphisms (SNPs) in the IL-13 gene were correlated with the long-term outcome of MCNS in Singaporean Chinese children (unpublished data). As IL-13 acts through the IL-4 receptor α subunit (IL-4RA), a common receptor with IL-4, this study aimed firstly to examine the genetic variations of IL-4RA in Singaporean Chinese children and, subsequently, to assess its association with atopy, steroid-response and long-term outcome in children with MCNS.

Materials and Methods

A total of 65 unrelated Singapore Chinese children with MCNS were included in this study. Their mean age at initial diagnosis was 4 years (range 1 to 14 years). According to the criteria from the International Study of Kidney Disease in Children (ISKDC),^{4,5} 33 children were defined as steroid-dependent, 25 were steroid-responsive (infrequent relapsers), while 6 were steroid-resistant. Clinical parameters of atopy, namely asthma, eczema, recurrent

TABLE I: GENOTYPE FREQUENCIES OF IL-4RA (EXON 11) POLYMORPHISM

Polymorphism	Genotype	Controls n (%)	MCNS n (%)	P value
E375A	EE	76 (83.5%)	51 (78.5%)	0.42
	EA	15 (16.5%)	14 (21.5%)	
	AA	0	0	
L389L (1417G/T)	GG	81 (89.0%)	55 (85.0%)	0.42
	GT	10 (11.0%)	10 (15.0%)	
	TT	0	0	
C406R	CC	75 (82.4%)	51 (78.5%)	0.54
	CR	16 (17.6%)	14 (21.5%)	
	RR	0	0	
L408L (1474T/C)	TT	75 (82.4%)	51 (78.5%)	0.54
	TC	16 (17.6%)	14 (21.5%)	
	CC	0	0	
R473H	RR	90 (98.9%)	63 (97.0%)	0.38
	RH	1 (1.1%)	2 (3.0%)	
	HH	0	0	
S478P	SS	75 (82.4%)	51 (78.5%)	0.54
	SP	16 (17.6%)	14 (21.5%)	
	PP	0	0	
Q551R	QQ	64 (75.3%)	46 (70.8%)	0.77
	QR	18 (21.2%)	17 (26.1%)	
	RR	3 (3.5%)	2 (3.1%)	
S727A	SS	90 (98.9%)	64 (98.5%)	0.83
	SA	1 (1.1%)	1 (1.5%)	
	AA	0	0	

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TABLE II: PAIRWISE LINKAGE DISEQUILIBRIUM BETWEEN IL-4RA SNPs AT EXON 11

	E375A	L389L	C406R	L408L	S478P	1902
E375A		109.31	134.63	141.00	141.00	19.61
L389L	0.83		105.08	105.08	105.08	13.39
C406R	0.97	0.80		141.00	141.00	23.02
L408L	0.97	0.80	1.00		141.00	23.02
S478P	0.97	0.80	1.00	1.00		23.02
Q551R	0.45	0.37	0.49	0.49	0.49	

Above diagonal, Δ ; below diagonal, χ^2 ; $\chi^2 > 9.49$, $p < 0.05$

TABLE III: REFERENCE ALLELE FREQUENCIES OF IL-4RA SNPs AT EXON 11 IN DIFFERENT CLINICAL COURSES

Clinical courses	E375A A (%)	L389 (1417G/T) G (%)	Q551R A (%)
Control	91.8	91.2	85.9
MCNS total	89.2	92.3	83.8
Steroids			
Responsive	92.0	94.0	90.0
Dependent	84.8	89.4	74.2 *
Resistant	100.0	100.0	100.0
Atopy			
With atopy	86.7	93.3	80.0
Without atopy	90.0	92.0	85.0
Outcome (≥ 5 years of follow-up)			
Complete remission	88.3	90.0	86.7
Relapsing	88.7	93.5	79.0

urticaria and allergic rhinitis, were noted in the children, of whom 15 were atopic. Of the 61 children who were followed up for at least 5 years, 30 were in complete remission, while 31 were still relapsing. 91 healthy Chinese children formed the control group. Informed consent was obtained from the parents prior to blood sampling, and this study had been approved by the Ethics Committee of National University Hospital.

Genomic DNA was extracted and screened for genetic variants of the IL-4RA gene using a direct polymerase chain reaction (PCR)-sequencing method. The exon 11, from which most of SNPs have been reported, was amplified by PCR. The primers were 5' CTGGAAAATCAGCATGGT 3' for sense, 5' TGGCTTGAG CTCTGAGCA 3' for anti-sense. PCR amplifications were carried out in 25 μ L volumes containing approximately 40 ng genomic DNA, 50 ng upstream or downstream primers, 200 μ M dNTP, 0.5 units Taq polymerase. The amplification procedure consisted of 35 cycles with the following parameters: denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1.5 minutes. Before sequencing, the PCR products were purified with QIAquick PCR purification kit (QIAGEN, Germany). ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kits (PE Applied Biosystems, USA) were then used for double-strand sequencing of the PCR products. Sequencing was performed on a GeneAmp PCR 9700 system (PE Applied Biosystems, USA). The products were then analysed on an Applied Biosystems Genetic Analyser 3100 (PE Applied Biosystems, USA).

Genotype frequencies were tested for the Hardy-Weinberg equilibrium using Pearson chi-square (χ^2) test, and the difference in the allele and genotype frequencies between cases and controls was analysed by χ^2 as well as Fisher's exact test. Linkage disequilibrium between different polymorphisms was assessed by delta (Δ). Statistical significance was defined as $p < 0.05$.

Results and Discussion

As in shown in Table I, we detected 8 SNPs in exon 11 of IL-4RA in Singaporean Chinese children, all of which were in Hardy-

Weinberg equilibrium. All these SNPs had been reported in other populations,⁶ but the genotype frequencies varied greatly. The reasons for the variation may be due to the different populations, distinct diseases studied as well as different standards for controls. As R473H and S727A were rare mutations in Singaporean Chinese, they were left out from further analysis. For the other 6 SNPs, we found that E375A, C406R, L408L and S478P were in almost complete linkage disequilibrium ($\Delta \geq 0.97$, $p < 0.01$), thus only E375A was used for further assessment (Table II). Our results did not demonstrate any significant difference in genotype (Table I) or allele (Table III) frequencies for E375A, L389L, Q551R, between the normal controls and MCNS in Singaporean Chinese children. These results are in agreement with reports from other populations,^{7,8} suggesting that these SNPs at exon 11 of IL-4RA do not predispose to MCNS.

We further stratified the patients into different subgroups according to steroid-responsive, atopy and long-term outcome (at least 5 years of follow-up), and evaluated their association with IL-4RA gene variation. As shown in Table III, we found that the Q551 allele frequency was significantly reduced in steroid-dependent MCNS, as compared to non-steroid-dependent MCNS (odds ratio 0.49, 95% CI 0.09-0.96, $p < 0.05$) and controls (odds ratio 0.59, 95% CI 0.25-0.99, $p < 0.05$). Among the SNPs of IL-4RA, Q551R has been the most frequently studied, and 551R allele has been found to be strongly associated with atopy⁹ and severe asthma¹⁰ in Caucasian populations. Functional studies suggested that 551R could produce a receptor with enhanced signal transduction *in vitro*.⁹

Conclusion

In this study on children with MCNS, we found that Q551R significantly correlated with steroid-response, with the 551R allele predisposing to steroid-dependency. These findings suggest that the genetic variants of IL-4RA, especially 551R may be used as a marker for the clinical course following steroid therapy in Singaporean Chinese children with MCNS. Further study needs to be done to elucidate the underlying mechanisms.

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Comparative Study of a Two-year Disease Management Programme for Patients with Diabetes in a Primary Healthcare Setting

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Introduction

Diabetes mellitus (DM) is the sixth commonest cause of death in Singapore in 2001¹ and is also the leading cause of end-stage renal failure and blindness in adults.¹ The crude prevalence of diabetes among adults in Singapore in 1998 was estimated at 9.0%.² Diabetes is also commonly encountered at the primary healthcare level and accounts for 7.9% of total cases seen in the National Healthcare Group Polyclinics in 2002.³

Primary care physicians play an important role in providing care for patients with diabetes. However, the majority of patients in Singapore with diabetes do not appear to have their condition adequately controlled, with 53% of patients having HbA1c of more than 8%.⁴

Evidence from trials had shown that with good glycaemic control, the development of microvascular complications could be delayed or prevented.^{5,6} A new strategy is needed to improve the diabetes control in patients in the primary care setting. Barriers to improving glycaemic control may be overcome by a system of collaboration between primary care physicians and diabetic educators.⁷ This is even more important in a busy primary healthcare setting where the contact time between the patient and the physician is limited.

In October 2000, the Ministry of Health, Singapore, piloted the Comprehensive Chronic Care Programme (CCCCP)⁸ at Choa Chu Kang Polyclinic from 1 October 2000. The polyclinic is a primary healthcare centre with an average of 194,000 attendances in 2001.⁹ As typical of a busy polyclinic, each doctor can only spend an average of a few minutes with each patient.

The programme consists of a structured, comprehensive protocol for care of patients with DM, hypertension and hyperlipidaemia. This programme was characterised by the following features:

- (1) Team-based approach for care of patients
The team consists of family physician, care manager, podiatrist and dietician
- (2) Structured clerking notes
Clinical notes are structured with reminders given at regular intervals for the doctor. Prompts are also present to suggest referral for poor control of targets
- (3) Structured notes and programme for care managers
Care managers are specially trained nurses who provide customised support care and monitor the progress of patients. Health education is individualised for each patients
- (4) Default tracking
Defaulters are tracked by the care managers and recalled if appointments are missed

A formative study suggested an improvement in DM control for diabetic patients in the CCCC group achieving a 2-point decrease in

HbA1c after 6 months as opposed to no change for the non-CCCC group.¹⁰ There was also an increase in patients achieving optimal diabetic control from 9.5% to 36.5%.

This is a follow-up study over a 2-year period to evaluate if the improvement seen at 6 months can be maintained and the overall effectiveness of such a disease management programme versus standard care.

Materials and Methods

The doctors in Choa Chu Kang Polyclinic referred patients with diabetes, hypertension and hyperlipidaemia into CCCC from October 2000. There was a tendency to enrol patients with more severe and complicated disease. The patients were assessed by the care managers and were enrolled into the programme if they were agreeable, making it an opt-in system.

Patients on the programme were classified into 4 different disease categories according to their disease severity, co-morbidities and complications. The categories are:

- Category 1 (Cat 1): On diet control only
Category 2 (Cat 2): On oral medication and/or insulin
Category 3 (Cat 3): With co-morbidities (hypertension and/or hyperlipidaemia)
Category 4 (Cat 4): With complications arising from diabetes

The initial programme started out identifying patients in all categories. For the purposes of our study, we only evaluated Cat 3 and 4 diabetic patients to concentrate on the patients with more serious disease.

We identified all Cat 3 or 4 diabetic patients who were enrolled in CCCC from 2 October 2000 to 30 April 2001. There were 164 patients (69.9% Cat 3 and 30.1% Cat 4) in the CCCC group. At the end of 24 months, 12 (7.3%) were followed-up at tertiary centres, 16 (9.8%) defaulted treatment, 8 (4.9%) were followed-up at other primary care facilities, 2 (1.2%) opted out of CCCC while 1 (0.6%) died. Five (3.0%) patients had incomplete data at the 24-month follow-up and were excluded.

They were compared with 183 Cat 3 or 4 patients who were not enrolled in CCCC (control). The patients in the control group were chosen retrospectively and matched for age. The same group of doctors and nurses managed them over the same period as the CCCC group. However, they were not part of any structured programme. These patients had been left out of CCCC entirely during the study period.

The measured outcomes were weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, HbA1c and low-density lipoprotein (LDL) at 0, 6, 12, 18 and 24 months.

The doctors took the blood pressures manually during each consultation by a sphygmomanometer. The weight and BMI were

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TABLE I: CHARACTERISTICS OF CCCP AND CONTROL POPULATION

	CCCP ¹ (n = 164)	Control (n = 183)	p
% Male (n)	43.9% (72)	42.6% (78)	0.810
Mean age (y)	58.5 ± 10.1	59.8 ± 9.0	0.194
Minimum age (y)	36	36	-
Maximum age (y)	78	78	-
% Insulin Dependent (n)	17.1% (28)	4.9% (9)	<0.001
% Hypertension (n)	68.3% (112)	78.1% (143)	0.038
% Hyperlipidemia (n)	76.8% (126)	69.9% (128)	0.148
% CVA ² /TIA ³ (n)	4.3% (7)	3.8% (7)	0.834
% Ischaemic Heart Disease (n)	11.0% (18)	9.8% (18)	0.728
% Category 3 (n)	70.1% (115)	66.1% (121)	0.425
% Category 4 (n)	29.9% (49)	33.9% (62)	-

¹CCCP: Chronic Comprehensive Care Programme; ²CVA: cerebrovascular accident; ³TIA: transient ischaemic attack

either recorded by the doctors or the care managers. HBA1c was performed in-house at the polyclinic by the same laboratory over the study period.

Statistical package used was SPSS for Windows Release 11.5.0. Chi-square test was used to evaluate significance of proportion and one-way analysis of covariance (ANCOVA) was used to evaluate the change in HBA1c.

Results

The selected characteristics of both groups at entry are shown in Table I. The CCCP and control groups were comparable in terms of age, severity classification, and presence of co-morbidity of stroke, heart disease and hyperlipidemia.

There were a higher proportion of CCCP patients on insulin compared to the control group ($p < 0.001$). In the CCCP group, 68.3% of patients had hypertension compared to 78.1% in the control group ($p = 0.038$).

The baseline mean HbA1c in the CCCP group was higher compared to the control group (9.8 ± 2.1 versus 8.2 ± 1.5) (Table II). However, the mean HbA1c were similar at 12 months for CCCP (8.0 ± 1.4) and control (8.2 ± 1.2). HbA1c showed a decrease of -9.8% (95%CI 12.9~-6.1) in the CCCP group and -0.8% (CI 3.1%~-1.6%) in the control group at 6 months. The two groups continued to show significant decrease beyond 12 months, on follow-up at 18 months (-8.0% for CCCP versus -4.0% for control, $p = 0.030$) and 24 months (-8.6% for CCCP versus -4.5% for control, $p = 0.030$).

TABLE II: AVERAGE VALUES ($\pm$ SD) OF 6 KEY PARAMETERS OF CCCP AND CONTROL POPULATIONS OVER 24 MONTHS

	Weight (kg)	BMI ²	Systolic BP ³ (mmHg)	Diastolic BP (mmHg)	HbA1c (%)	LDL ⁴ (mmol/L)
0 month						
CCCP (n = 163)	65.1±11.8	26.0±4.3	136.1±17.8	81.1±8.6	9.8±2.1	3.9±1.2
Control (n = 183)	66.5±12.0	27.0±3.7	136.1±15.5	80.4±7.4	8.2±1.5	3.7±1.0
6 month						
CCCP (n = 142)	64.6±11.5	25.7±4.1	132.6±13.0	79.9±6.8	7.9±1.6	4.1±0.8
Control (n = 183)	70.0±16.5	29.4±5.4	131.4±12.1	78.7±7.7	8.3±1.6	3.7±0.8
12 month						
CCCP (n = 128)	64.9±12.7	26.0±4.6	135.0±15.9	78.8±7.4	8.0±1.4	3.6±0.9
Control (n = 183)	67.5±13.8	27.5±4.7	136.1±15.6	79.2±8.6	8.2±1.2	3.6±0.9
18 month						
CCCP (n = 130)	65.7±13.4	25.9±4.7	134.4±14.6	78.8±7.7	7.7±1.3	3.7±0.9
Control (n = 183)	69.4±11.2	28.8±3.6	138.5±15.3	80.0±8.2	8.0±1.2	3.7±0.9
24 month						
CCCP (n = 117)	64.2±11.2	25.0±4.9	134.6±11.1	77.7±7.5	7.6±1.1	3.5±0.8
Control (n = 183)	61.8±7.7	22.5±8.5	136.3±15.2	78.7±7.4	7.9±1.2	3.4±0.9

¹CCCP: Chronic Comprehensive Care Programme; ²BMI: body mass index;

³BP: blood pressure; ⁴LDL: low-density lipoprotein

TABLE III: ONE-WAY ANALYSIS OF COVARIANCE (ANCOVA) TEST FOR HBA1c

	Mean change (95%CI ¹)		p
	CCCP ²	Control	
6 months (n = 111)	-9.8% (-12.9 ~ -6.1)	-0.8% (-3.1 ~ 1.6)	<0.001
12 months (n = 104)	-6.2% (-9.7 ~ -2.8)	-3.2% (-5.8 ~ -0.6)	0.182
18 months (n = 107)	-8.0% (-10.7 ~ -5.2)	-4.0% (-6.1 ~ -1.9)	0.030
24 months (n = 95)	-8.6% (-11.5 ~ -5.6)	-4.5% (-6.6 ~ -2.3)	0.030

¹CI: confidence interval; ²CCCP: Chronic Comprehensive Care Programme

One-way ANCOVA was not significant for weight, BMI and LDL levels.

Discussion

It has been estimated that annual per capita healthcare expenditure in the United States in 1997 was 4-fold for those with diabetes compared to the general population.¹¹ Good diabetes control can reduce complications, improve quality of life for patients and ultimately reduce the economic burden on the country.

With a rapidly ageing population and prolonged life expectancy,¹² the prevalence of diabetes is expected to increase in Singapore over the next few years. A sedentary lifestyle and changes in dietary habits also contribute to the worsening problem.

Diabetes is a chronic condition where long-term lifestyle modifications are necessary for control of illness. Care of patients should consist of education, motivation of patients and support for patients. As a significant proportion of diabetic patients are followed-up in primary healthcare, it is important to provide structured care, regular HBA1c checks to assess control and screening for complications.

A team-based approach to managing diabetes is especially important at the primary care level due to the amount of time a doctor can spend with the patient. A disease management programme provides a systematic and comprehensive care for our chronic patients. A multi-faceted disease management programme for diabetes can result in significant improvement in diabetic care.¹³ A disease management programme can lead to significant reduction in healthcare costs and other measures of healthcare use.¹⁴

Patients enrolled on our programme can discuss management issues with our care managers during their regular follow-ups together with telephone assess. Our care managers also conduct workshops, lectures and organise support groups. This constitutes an important component of education to increase patients' motivation and provides them with necessary knowledge for self-care.

Glycated haemoglobin also provides useful information on health status and future medical charges for patients.¹⁵ The results from our study show that HBA1c levels for patients on CCCP decrease by a mean of 8.6% compared to 4.5% for the control group.

The mean blood pressure change for both groups was not significant. However, the mean blood pressures in both groups were already low to begin with.

The benefits to diabetic patients can be observed from the results of our study. A decrease in HBA1c levels will ultimately lead to lower complications from diabetes and improved quality of life for our patients.

The limitation of this study is that we were unable to demonstrate significant improvement in weight loss and change in LDL levels. The challenge for us in the future is to fine-tune our programme to facilitate weight loss and decrease LDL levels in our diabetic patients.

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Special Poster Presentation

Tissue Microarray Study for Classification of Breast Tumours

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Introduction

Breast cancer is one of the most common malignancies and a leading cause of death among women. The sporadic (non-inherited) breast cancer that constitutes more than 90% of all breast cancer is a complex and heterogeneous disease, at both the clinical and molecular levels. Genetic changes resulting in altered mRNA and protein levels, such as the activation or amplification of oncogenes or the loss of tumour suppressor genes, have been described in breast tumorigenesis. The clinical and pathological heterogeneity of breast cancer hinders selection of appropriate treatment for individual cases exclusively based on the histopathologic status, such as tumour grade, stage and type. Recently, breast tumours have been classified by their gene expression profiles at gene or protein levels, and the variation in gene expression patterns provide a distinctive molecular portrait of each tumour or closely related group of tumours. This novel tumour taxonomy elucidates the biological variance of tumours and provides better insight into the complex biological process involved in tumorigenesis. The validation of such a classification on formalin-fixed paraffin-embedded tumour archives linked to clinical information in a high-throughput fashion would have a major impact on clinical practice. In this study, we aimed to test the ability of tumour tissue microarrays to sub-classify breast cancers based on the immunohistochemical profilings of biomarkers. Subgroups with high malignant potential will also be assessed.

Materials and Methods

A tumour tissue array and a normal tissue array were constructed from 97 tumours and corresponding non-tumour tissues, using a tissue-arraying instrument. A tumour array was composed of 97 tumours and 5 normal tissues (as internal control) and a normal tissue array consisted of 97 matched normal tissues and 5 tumour tissues (as internal control). Of the 97 tumours, 11.3% had a histological grade 1, 39.2% a grade 2 and 49.5% a grade 3. Consecutive 4 µm-thick TMA sections were cut and placed on charged polylysine-coated slides for immunohistochemical analyses. A panel of 7 important biomarkers (ER, PR, c-erbB2, Cox-2, p53, VEGF and PDGF) was applied to representative sections of the tissue arrays. Standard indirect immunoperoxidase procedures were used for immunohistochemical staining. Briefly, tissue microarray sections were deparaffinised and treated with 3% hydrogen peroxide in phosphate-buffered saline to block endogenous peroxidase activity. For ER, PR, c-erbB2 and p53, the sections were treated with an antigen retrieval solution (10 mM citric acid monohydrate, pH 6.0), which was preheated at 95°C for 10 minutes. For Cox-2, VEGF and PDGF, the sections were subjected to heat-induced epitope retrieval in a microwave oven using the DAKO® Target Retrieval Solution (DAKO Corporation, Carpinteria, CA, USA), according to the manufacturer's instructions. Monoclonal antibodies against the protein biomarkers were used as primary antibodies. All the sections

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were incubated with the respective primary antibody for 1 hour at room temperature, followed by the HRP-labelled polymer for 30 minutes and DAB+ substrate-chromogen solution for 5 minutes (DAKO EnVision⁺ System, DAKO). All stained sections were counterstained with haematoxylin, dehydrated and mounted. The density and percentage of the stained tumour cells were evaluated for different biomarkers and expression levels were graded as negative, weak, moderate and strong. A tumour with moderate to strong staining was considered as positive. The tumours were classified using a monothetic (binary variables) clustering algorithm (S-plus 2000 software). Pearson's chi-square test with Yate's continuity correction was used for statistical analysis. A p value of <0.05 was considered statistically significant

Results

Based on the intensity and percentage of stained tumour cells, 47 of 95 cases were Cox-2 positive and 31 of 93 cases were p53 positive. Positive staining is found in 53 cases for ER, 54 cases for PR and 30 cases for c-erbB2. VEGF and PDGF were overexpressed in 55 of 89 cases and 58 of 90 cases, respectively. Using the monothetic clustering algorithm, 2 distinct groups of tumours are characterised by the oestrogen receptor (ER) status and tumour grade. Group I included tumours with ER negative, 75% of them with a grade 3; whereas group II consisted tumours with ER positive, 52% of them with a grade 2 ($p = 0.0026$). Four biomarkers, c-erbB2, Cox-2, p53 and VEGF, were significantly overexpressed in group I; with statistically significant difference. There were no significant differences for p53 ($p = 0.079$) and PDGF ($p = 0.497$) expression between the two groups. Each group of tumours was further subgrouped based on the

VEGF status, and 8 subsets of tumours were identified according to the expression status of c-erbB2 and p53. These 8 subsets were defined as ER-/VEGF+/c-erbB2+, ER-/VEGF+/c-erbB2-, ER-/VEGF-/p53+, ER-/VEGF-/p53-, ER+/VEGF+/p53+, ER+/VEGF+/p53-, ER+/VEGF-/p53+ and ER+/VEGF-/p53-. The correlations of these biomarkers were group dependent. The direct association of p53 with c-erbB2 was found in group I ($p = 0.048$). Co-expression of Cox-2 with VEGF was mostly found in subgroup ER-/VEGF+ rather than in subgroup ER+/VEGF+ ($p = 0.006$). c-erbB2 was significantly co-expressed with either Cox-2 or VEGF in subgroup ER-/VEGF+, not in subgroup ER+/VEGF+ ($p = 0.011$ and $p = 0.015$, respectively). The malignant potential of the ER-/VEGF+ subgroup was associated with the strong correlations of Cox-2 and c-erbB2 with VEGF.

Conclusion

Our results indicate that this molecular classification system, based on the statistical analysis of immunohistochemical profiling, is a useful approach for tumour grouping. The created subgroups may represent more homogeneous disease entities with definable genetic mechanism and may allow further study of specific genetically-controlled metabolic pathways. This approach may hold great promise in identifying biologically homogeneous subgroups in which molecular interactions can be easily addressed. The tissue microarray-based classification with limited protein biomarkers can sub-classify tumours into clinically and biologically relevant subgroups, and rationalise the application of different therapeutic strategies for different subgroups of breast.

Poster Presentation Abstracts – Day One

I/II – Day One/Day Two; xxx – Poster Board Number; GP: General Practice; MP – Medicine/Paediatrics; NUR – Nursing; PD – Paramedical Disciplines; SOGD – Surgery/Obstetrics & Gynaecology/Dentistry

1001/GP

State of Health of Community-Dwelling Elderly in Singapore – The Challenges Ahead

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Aim: To assess the state of and identify the health issues facing community-dwelling elderly in Singapore.

Methods: 1954 households were randomly selected in Toa Payoh Central, a matured housing estate with approximately 9000 households and an elderly prevalence of 11%. 1092 households (47%) were without elderly and a 1-step replacement was performed. A total of 231 households and 265 elderly participated, with 229 households declining participation, giving a household response rate of 50.2%. The study was conducted by a team of medical students in the elderly's homes. Elderly were administered a questionnaire which included basic activities of daily living (ADL, using Barthel Index), instrumental ADL, living conditions, social and medical history. Physical measurement taken includes height, weight, blood pressure, timed 'Up and Go', and mental state examination.

Results: Subjects' mean age was 72.7 ± 6.1 years. The elderly were mostly women (57.0%) and had less than 7 years of education (77.7%). Only 10.6% lived alone. Most were ADL independent (72.5%; 95% CI 67.1-77.9), and urinary incontinence and ability to climb stairs were the commonest function affected. Instrumental ADL independence was 62.6% (95% CI 56.8-68.4). Fifty percent of the elderly had Body Mass Index (BMI) ≥ 23 . The commonest chronic conditions affecting the elderly were hypertension (58.6%), arthritis (45.5%) and diabetes mellitus (23.5%).

Conclusion: While elderly studied were mostly functionally independent, the high BMI and high prevalence of lifestyle diseases like diabetes and hypertension relative to available data of elderly in other countries, will pose an enormous challenge ahead.

1002/GP

Improved Molecular Diagnostic Assays for Common Non-deletional Southeast Asian α - and β -Thalassaemia Mutations

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Aim: The thalassaemias are the most common monogenic disorders in the world, and are endemic to Southeast Asia. We previously developed a multiplex-minisequencing strategy to screen for the common nondeletional α - and β -thalassaemia mutations. Although faster and less laborious than other methods, reagent usage was high and one of the mutations (D619bp) required separate agarose gel analysis. We now describe an improved assay.

Methods: PCR amplifications were performed as described previously. Multiplex-minisequencing was performed in a total reaction volume of 2 ml. Minisequencing primers were designed to anneal next to each mutation site, and comprised a total of 7 common α -thalassaemia mutations (codon 0D1bp, Constant Spring, Paks, Quong Sze, Suan Dok, codon 30D3bp, codon 59) and 16 common β -thalassaemia mutations (Panel I: codon 41/42, IVSII nt654, IVS1 nt5, codon 17, -28, -29, codon 71/72, codon 26; Panel II: IVSInt1, codon 19, codon 0, codon 43, codon 27/28, codon 8/9, codon 35, and D619bp). Products were subjected to capillary electrophoresis on an ABI PRISM[®]3100, and analysed using GeneScan[™] and Genotyper[™]3.7 software.

Results: All mutations in all homozygous wild-type, homozygous mutant, heterozygous, and compound heterozygous samples were correctly identified using the ABI 3100. The results obtained were indistinguishable from those using the old protocol.

Conclusion: We have developed an improved, simpler and less expensive thalassaemia molecular screening assay based on multiplex-minisequencing and capillary electrophoresis. The 5-fold reduction in reagent usage resulted in a 47% reduction in per sample test costs (SGD10 vs SGD17.50) excluding manpower. The use of a single test platform for screening all mutations further simplified test procedure and reduced manpower utilisation.

1003/MP

Effective Protective Measures for Healthcare Workers in an Intensive Care Unit Dedicated for Patients With Severe Acute Respiratory Syndrome (SARS)

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Aim: A retrospective study to document the number of healthcare workers (HCW) developing severe acute respiratory syndrome (SARS) after the implementation of protective measures in an intensive care unit dedicated for patients with SARS.

Methods: Data was collected for the period of 17 March to 27 April 2003. The diagnosis of SARS was made by Infectious Disease Specialist using prevailing case definitions recommended by the World Health Organisation. The exposure risk of all the HCW in the ICU were evaluated in terms of working hours and procedures performed on patients. The protective measures were strictly applied for all HCW through organised training and regular checks. Surveillance of the staff (and their families) developing illness was effective through a centralised disease screening and reporting system.

Results: During the study period, a total of 230 HCW worked in the SARS ICU, during which 42 patients with probable SARS were progressively admitted, resulting in daily occupancies ranging from 1 to 21. One of the patients was admitted on 12 March 2003, prior to the recognition of the syndrome and the full implementation of protective measures. 19 HCW developed SARS through unprotected contact with her. Subsequent to the implementation of the protective measures, no other ICU HCW developed SARS for a period of 52 days.

Conclusion: The data in this paper demonstrated the effectiveness of the protective measures practiced in a SARS ICU. However, persistent personal vigilance in proper practice for these measures a crucial factor in its continued effectiveness.

1004/MP

The Effect of Clozapine on Brain-derived Neurotrophic Factor Distribution in the Rat Brain

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Aim: The aim of this study is to investigate the effects of acute clozapine treatment on the pattern and intensity of BDNF immunoreactivity in the rat brain, and hence the effects of D4-receptor blockade on this important neurotrophin.

Methods: A cohort design using adult Wister rats was used. At the end of the experimental period, the animals were sacrificed, transcardiac perfusion of their brains carried out, followed by removal and cryoprotection. Immunological procedures using polyclonal rabbit anti-BDNF antiserum and biotinylated goat anti-rabbit antibody were carried out. Finally, the specimens were treated with Avidin-Biotin complex and Diaminobenzidine, mounted on slides and examined under light microscopy.

Results: The results of this study show that acute blockade of the dopamine D4-receptor with clozapine exerts dramatic effects on BDNF immunoreactivity in the mesolimbic pathway and its' target regions. There was also reduced immunolabelling in the cell bodies in the extended amygdala, hippocampus, midbrain and parts of the limbic cortex after clozapine treatment.

Conclusion: The primary findings in this study show the ability of dopamine D4-receptor blockade to regulate the production of BDNF. Clozapine's effect on BDNF expression via the D4-receptors most likely contributes to its effectiveness as an antipsychotic agent, whether directly or indirectly via other neurotransmitters or second messenger systems. This may explain why it is effective in the treatment of refractory schizophrenics, who do respond to conventional D2-receptor blockade.

I005/MP**Nuclear Cardiology as the Gatekeeper – Utilisation Patterns in National University Hospital**

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Aim: Nuclear cardiology is recognised as a valuable tool for cardiac risk-stratification. The utilisation patterns of the technique in Southeast Asia have not been previously reported. Also, the prognostic value of a normal Tc-99m tetrofosmin SPECT scan in a large Asian population has not been well established.

Methods: A consecutive series of 4512 patients underwent stress Tc-99m tetrofosmin SPECT imaging over 3 years (April 1999 to July 2002) at the National University Hospital, Singapore. Uniform methods of data collection and standardised epidemiology methods for follow-up were employed. Follow-up was complete in 91% of patients.

Results: The average age was 60 years; 2585 (57%) were male. 60% had exercise stress testing. 2899 (64%) had a normal stress scan. 11 (0.004%) of these patients with normal scans underwent angiography within 3 months of the index myocardial perfusion imaging (MPI) study, of which 8 had significant lesions. At 12 months of follow-up, 0.5% of patients with a normal scan had experienced a cardiac event (myocardial infarction/cardiac death). 1613 patients had abnormal scans, of which 137 underwent angiography within 3 months after the index study. Patients with abnormal scans (n = 766) did not undergo angiography for the following reasons: small defect (22%); patient-request for medical therapy only (22%); elderly (12%); concomitant renal impairment (11%).

Conclusion: 1) The negative predictive value of a normal Tc-tetrofosmin stress-test in this Asian population was very high and conferred excellent cardiac prognosis. 2) The technique is utilised by physicians for cardiac risk-stratification in both patients with newly-diagnosed and established coronary artery disease.

I006/MP**Prevalence of Mitral Regurgitation Following ST-Elevation MI in Patients Treated with PPCI Compared to Thrombolytic Therapy**

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Aim: The presence of mitral regurgitation (MR) post ST-elevation myocardial infarction (STEMI) is associated with a poorer prognosis. We wish to examine if primary percutaneous intervention (PPCI) has any effect on the prevalence of MR compared to thrombolytic therapy.

Methods: We studied 402 consecutive patients admitted to our institution with a diagnosis of first STEMI from August 2001 to May 2003. MR was classified semi-quantitatively on transthoracic echocardiography into none, mild, moderate or severe. Those with mitral valve prolapse were excluded.

Results: The site of STEMI was anterior in 239 patients (59.5%) and 161 (40%) had PPCI while 160 (40%) received thrombolytic therapy; the remainder had conservative treatment. Median time of echocardiography was 3 days post STEMI. MR was more common and severe (p = 0.0008, p = 0.04) in inferior STEMI as out of 163 patients, it was mild in 85 (52%), moderate in 12 (7%) and severe in 1 (1%) patients. In 239 patients with an anterior STEMI it was mild in 95 (40%) and moderate in 8 (3%). There were no differences in the prevalence of MR regardless of therapy given. Patients with MR had a poorer ejection fraction (EF) (43% vs 46%, p = 0.029) but no differences in the creatinine kinase (CK), CK-Mb or Troponin-T.

Conclusion: MR is more common in inferior STEMI but the prevalence post STEMI is not reduced by choice of therapy. MR appears to be associated with a poorer EF, this may explain the poorer prognosis seen in other studies.

I007/MP**Predictors of Early- and Long-Term Mortality for Patients Undergoing Modern Primary Angioplasty for AMI**

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Aim: Primary percutaneous coronary intervention (PPCI) has been shown to be an effective and superior reperfusion therapy for patients with acute myocardial infarction (AMI). The strategy has undergone dramatic evolution with the introduction of coronary stent, adjunctive glycoprotein (GP) IIb/IIIa

inhibitor and thrombectomy devices. The goal of this study is to examine the outcomes of PPCI and to identify risk factors for early- and long-term mortality.

Methods: Our institution's angioplasty database was used to identify patients who underwent PPCI within 6 hours of AMI without antecedent fibrinolytic therapy. From January 1997 to June 2002, a total of 216 consecutive and unselected patients who underwent PPCI were analysed for this study. Multivariate analyses were performed to identify significant risk factors for short- and long-term survival.

Results: The patient cohort comprised 85% males and the mean age of all patients was 56.2 ± 12.2 years. Diabetes mellitus was a risk factor in 29%. The types of PPCI procedure carried out were: coronary stenting in 99%, adjunctive use of GPIIb/IIIa in 41%, thrombectomy in 18% and distal protection device 0.5%. Cardiogenic shock was present in 21 (10%) patients. The in-hospital mortality rate for all PPCI patients was 10.7% (95% CI 6.8%, 15.5%). This rate dropped to 5.4% (95% CI 2.7%, 9.4%) when deceased patients with pre-procedural cardiogenic shock were excluded. Cumulative mortality rates for all patients at 6 months and 1 year were 12.0% (95% CI 8.0%, 17.1%) and 13.0% (95% CI 8.8%, 18.2%), respectively. Recurrent cardiac events (angina, reinfarction and congestive cardiac failure) rate at 6 months and 1 year were 20.3% (95% CI 14.7%, 27.0%) and 26.1% (95% CI 17.3%, 38.2%), respectively; and repeat revascularisation rates were 7.9% (95% CI 4.4%, 12.9%) and 10% (95% CI 4.9%, 19.1%), respectively. Multivariate analyses identified age (>75 years) and triple-vessel coronary artery disease as significant predictors of early and late mortality. Neither gender nor diabetes mellitus was predictors of outcomes.

Conclusion: Primary PCI is a highly effective treatment strategy for AMI. Adverse cardiac events tend to occur early within 6 months and diminish at 1 year. Old age and multi-vessel coronary artery disease are predictors of poor outcomes.

I008/MP**Evaluation of Occlusive Balloon Distal Protection Device For Prevention of Distal Embolisation During PCI**

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Aim: Embolisation of atherothrombotic debris during percutaneous coronary intervention (PCI) is associated with significant increase in periprocedural major adverse cardiac events (MACE). We evaluate the safety and effectiveness of an occlusive distal protection balloon (GuardWire Plus) designed to retrieve plaque material dislodged during PCI in preventing distal embolisation.

Methods: Between June 2001 and March 2003, GuardWire device was employed in 44 patients undergoing PCI in our centre. The procedure was performed in native coronary arteries (n = 39) and degenerated saphenous vein grafts (SVGs) (n = 5) in patients with ST-elevation MI (36%), unstable angina/non-ST elevation MI (50%) and stable angina (14%). The study endpoints were clinical efficacy of the device and MACE during in-hospital and at 30 days.

Results: The patient cohort comprises 86% male with a mean age of 54 ± 10 years. The mean lesion length was 15.7 ± 6.0 mm and thrombus was present in 75%. Coronary stenting was performed in 100% of cases. Glycoprotein IIb/IIIa inhibitors were administered in 14/44 (32%) patients. The GuardWire device was successfully deployed and retrieved in all patients. The pre-PCI flow (57% TIMI 0-1) was improved procedurally (95% TIMI 3) with the device. The in-hospital MACEs were: 2/44 (4.5%) nonQ-MI, 1/44 (2.3%) stroke and 0/44 (0%) death. Device complications include: coronary perforation in 2/44 (4.5%), distal thromboembolism 1/44 (2.3%) and vascular complications 2/44 (4.5%). At 1 month, there was 1 death but no recurrent MI or repeat revascularisation.

Conclusion: This preliminary experience indicated that the GuardWire system is safe and effective in reducing atheroembolism during PCI in native coronary vessels and SVGs. The MACE rates appear to compare favourably with published data.

I009/MP**MI in Young Adults: Comparative Analysis of Risk Factor Control**

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Aim: This study compares the quality of care of hypertension, diabetes and dyslipidaemia between young (defined as age <45 years) and older patients with acute myocardial infarction (AMI).

Methods: 1867 patients were admitted to the National University Hospital with AMI from 2000 to 2002. We compared the proportion of patients with known but untreated risk factors prior to MI in those <45 years old (n = 235) and those ≥45 years old (n = 1632).

Results: 30% of those <45 years old with hypertension were untreated prior to admission compared to only 10% in those ≥45 years old; odds ratio (OR) 4.0 (2.4-6.5)*. 13% of known diabetics <45 years old were not on treatment compared to only 5% (30/659) in those ≥45 years old; OR 2.7 (1.1-6.9)*. 30% of known dyslipidaemics <45 years old were not on treatment compared to 12% of those ≥45 years old; OR 2.9 (1.6-5.2)*.

* adjusted for previous episode of MI which was 6% (<45 years) vs 17% (≥45 years); P was <0.0001 for all comparisons.

The 28-day mortality was 10% (24/235) for <45 years old vs 19% (315/1632) for ≥45 years old (odds ratio 2.1, 95% CI 1.7-4.0, p = 0.0005).

Conclusion: Control of the 3 major risk factors was uniformly and significantly poorer in those <45 years old. Singaporean males serving national service undergo a mandatory comprehensive health screening at the age of 35 years old. This provides an important opportunity for coronary disease prevention as 90% of patients under 45 years old were male.

1010/MP

Simple Echocardiographic Doppler Variables that will Predict Tricuspid Regurgitation Severity

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Aim: Currently, there is no simple reliable method for assessment of tricuspid regurgitation (TR) severity. We evaluated the utility of simple, easily derived Doppler of right ventricular filling variables to measure TR severity.

Methods: A prospective analysis of 44 consecutive patients with TR, classified into mild, moderate or severe by echocardiographic evaluation using colour Doppler. Tricuspid inflow Doppler variables of peak E wave velocity, peak A wave velocity, E/A ratio, E wave deceleration time, and the right ventricle tissue Doppler variables of E' velocity, A' velocity, E'/A' ratio and systolic velocity were analysed.

Results: Severity of TR was associated with an increase in the peak E wave velocity (p < 0.001) and E/A ratio (p < 0.001), a decrease in E wave deceleration time (p < 0.001). The right ventricular tissue Doppler variables showed a decrease in A' velocity (p < 0.001), an increase in the E'/A' ratio (p = 0.0026) and the E/E' ratio increases in proportion to TR severity (p < 0.001). Severe TR can be identified with a peak E wave velocity > 0.7 m/s (sensitivity 100%, specificity 100%), E/A ratio > 2.5 (sensitivity 94%, specificity 100%), E wave deceleration time < 160ms (sensitivity 100%, specificity 92%) and E/E' ratio > 7 (sensitivity 89%, specificity 100%).

Conclusion: Tricuspid inflow Doppler and right ventricular tissue Doppler variables are easily obtainable and useful in the evaluation of tricuspid regurgitation severity. Peak E wave velocity of > 0.7 m/s is the best predictor of severe TR.

1011/MP

Prevalence of Emotional Symptoms and the Risk of Violence in Adolescents with Psychosis and Schizophrenia

Say How ONG

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Aim: Psychotic illness in adolescents are relatively rare compared to other psychiatric diagnoses. The relationship of emotional symptoms such as anxiety, depression and obsessive compulsive disorder (OCD) with childhood psychosis is not very much known. This study attempts to examine this and assess the risk of violence and suicide ideations among these adolescents.

Methods: Adolescents (n = 13) who had been admitted to the Child and Adolescent Inpatient Unit of Woodbridge Hospital in year 2002 to 2003 were screened for psychosis and schizophrenia. The diagnosis must satisfy both the DSM-IV Criteria and K-SADS. A similar group of adolescents (n = 18) with the diagnoses of anxiety, depression or OCD was used as a control group.

All parents were given the Child Behaviour Checklist (CBCL) while the adolescents filled self-reported questionnaires on anxiety (MASC and SASC-R), depression (CDI) and suicide ideations (SIQ). The clinicians assessed their functioning using GAF scale and risk of violence with SAVRY.

Results: The subjects have a poorer GAF score with a mean of 47.31 ± 7.85 vs 58.06 ± 10.82 (p < 0.05) for control group. The 2 groups do not differ in their anxiety scores though the control group have a higher raw score in their negative self evaluation (p < 0.05). The subjects and controls do not differ in their score for suicidal ideations and risk of violence.

Conclusion: This study suggests that psychotic teenagers are not less vulnerable to emotional symptoms such as anxiety. Both groups do not differ in their suicide ideations. Adolescents with psychosis and schizophrenia are no more prone to violence than their anxious or depressed counterparts. Clinicians should pay more attention to the emotional symptoms of psychotic adolescents.

1012/MP

A Preliminary Description of Children with Attention Deficit Hyperactivity Disorder (ADHD) in Singapore

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Aim: ADHD is the most common diagnosis in the Child Guidance Clinic, the main child mental health facility in Singapore. This study aims to develop a systematic assessment procedure for ADHD in a mental health facility in Singapore.

Methods: A total of 27 children with a clinical diagnosis of ADHD were invited to participate in this study. Parents were administered the Child Behavior Checklist for Children, the SNAP (SWANSON) scale, the ADHD Rating Scale-IV and the Diagnostic Interview Schedule for Children-IV. School teachers were given the Teacher Report Form to complete. The children were assessed using the Wechsler Intelligence Scale for Children-III, the British Picture Vocabulary Scale-II, the Neale Analysis of Reading Ability-II, the Wide Range Achievement Test-3, as well as the Conner's Continuous Performance Test-II.

Results: General screening measures were not useful in identifying ADHD compared to using specific screening tests. The CPT-II was able to predict ADHD in only about half of the cases. More than 80% of the cases also met criteria for ADHD using the DISC IV, suggesting that clinical diagnoses closely correlated with a structured interview of parents. The overwhelming majority of children had average and above average intelligence and less than 25% had evidence of learning disabilities. Among co-morbid conditions, oppositional defiant disorder was present in about 50% of cases although none of them fulfilled criteria for conduct disorder.

Conclusion: ADHD is a distinct diagnostic entity in Singapore. More studies are needed to understand this group of children.

1013/MP

Bedside Fiberoptic Bronchoscopy is a Safe Procedure if Done in the Paediatric Intensive Care Unit under Conscious Sedation

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Aim: Bedside fiberoptic bronchoscopy is a valuable tool in the diagnosis and treatment of various respiratory conditions in critically ill patients. This procedure is performed in our paediatric intensive care unit under conscious sedation. We examined complications that occurred during this procedure.

Methods: We retrospectively reviewed 107 procedures performed on 48 patients between March 2000 and November 2001. All patients were continuously monitored with cardiorespiratory and pulse oximetry monitors. Patients either received blow-by aerosolised oxygen or positive pressure ventilation via an endotracheal tube. The flexible bronchoscope used was the Olympus fiberoptic bronchoscope (model no. BFXP40) with a 2.8-mm outer diameter.

Results: The median age of the patients was 29 months (range 1 to 306 months). 55% (n = 59) of the procedures were done via the endotracheal tube, 40% (n = 43) were performed transnasally while 5% (n = 5) were done via a tracheostomy. The commonest complication was transient desaturation during the procedure. Only 6 patients (5.6%) developed transient desaturation. These patients responded to bag and mask ventilation if they were not

intubated. Those on the ventilator required transient increase in ventilator settings. 1 patient developed haemorrhage from the inflamed mucosa after suctioning. 1 patient developed transient post-procedure stridor secondary to laryngeal oedema. The remaining 2 patients had complications from sedation rather than the procedure.

Conclusion: Bedside flexible fiberoptic bronchoscopy under intravenous conscious sedation in children in the paediatric intensive care unit is safe. Complications are uncommon and if they occur, they are easily corrected. Proper monitoring and trained personnel are however important to avoid potential complications.

I014/MP

Can Histopathological Features of Breast Carcinoma Alone, Independent of Lymph Node Status, Predict a Positive Bone Scan Yield?

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Aim: To improve cost-effectiveness, some recommend staging bone scintigraphy be performed in breast cancer of higher TNM stages only. Sentinel lymph node mapping and associated surgical management impact upon these recommendations and ultimately, upon when to perform bone scintigraphy. We aim to confirm a direct positive correlation between histological grading and bone scan results, and ascertain if predictability of a positive yield of bone scintigraphy can be determined by assessment of histopathological grading of the primary tumour alone.

Methods: 120 consecutive staging bone scans for breast carcinoma were categorised as 'no metastases' or 'definite skeletal metastases' without knowledge of histopathological grading. 'Possible metastases' results were correlated with other prior/follow-up imaging and/or clinically and recategorised into either the 'no metastases' or 'definite metastases' group. Post-mastectomy histopathologic grading (the Bloom-Richardson system for infiltrative/invasive tumours and nuclear grading for in-situ tumours) was correlated with bone scan results and axillary lymph node status. Bone scan results was correlated with nodal status and size of tumour.

Results: a) Higher histopathological grading correlates with bone scan positivity, b) Positive axillary lymph node status correlates bone scan positivity, c) Histopathological grading does not predict with lymph node positivity, and d) Size of primary tumour does not predict bone scan positivity.

Conclusion: Higher histopathological grading and positive nodal status independently correlate with bone scan positivity. Grade 3 is most likely to have a positive bone scan. Histopathological grading is a better predictor than tumour size. The importance of this study lies in assisting clinical decision for bone scanning in breast carcinoma staging.

I015/MP

Therapeutic Drug Monitoring of Low-dose Paclitaxel as Radiation Sensitizer in Human Plasma Using Highly Sensitive Reversed-phase High-performance Liquid Chromatography

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Aim: Recently, there is growing interest in using paclitaxel as a radiation sensitizer. The increased cell radiosensitivity with exposure to paclitaxel is evident at drug levels much lower than the concentrations required for cytotoxic effects. In this case, the current available HPLC-UV methods would be not sensitive enough to measure all the levels of paclitaxel in patients' plasma. In this study, a highly sensitive HPLC-UV method was developed and validated.

Methods: Paclitaxel concentrations were quantified using 0.5 ml of human plasma with docetaxel as the internal standard. The sample purification was carried out through solid-phase extraction. Good chromatographic separations were achieved using a Nova-Pak C18 column (3.9 mm, 300 mm, 4 mm). The optimised mobile phase consisted of methanol and phosphoric acid solution (pH = 2.6) (70:30). Paclitaxel and internal standard were eluted with retention times of 9.5 and 11.2 min, respectively.

Results: An excellent linearity was achieved over the range of 5 to 200 ng/ml with a mean correlation coefficient (r²) of 0.9993. The limit of detection was 2 ng/ml. The accuracy (deviation) was within 12% for all quality control samples, and the precision (RSD) varied from 1.4% to 9.8%. This highly sensitive HPLC-UV method was further confirmed using tandem LC-MS assay which is 10 fold more sensitive than HPLC-UV.

Conclusion: The method has been successfully applied to determine all plasma samples with the lowest concentration of 6.8 ng/ml from phase II study of intensified concurrent chemotherapy (paclitaxel 20 mg/m²/day) and conventional radiotherapy in the treatment of locally advanced nasopharyngeal carcinoma.

I016/MP

Aneurysm Detection on Magnetic Resonance Imaging

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Aim: The 3 Tesla (T) magnetic resonance (MR) scanners yield improved signal to noise ratio and spatial resolution compared to the 1.5 T scanners. The improved resolution potentially enables MR angiography (MRA) at 3T to rival catheter angiography for evaluation of intracranial vasculature. We report our initial experience with unenhanced 3D time-of-flight (TOF) MRA in the detection of unruptured intracranial aneurysms.

Methods: From an eligible population of 850 patients who underwent unenhanced 3D TOF MRA on a 3 T MR scanner, 10 patients with unruptured intracranial aneurysms were retrospectively identified. Seven patients underwent catheter angiography and 2 patients had previous 1.5 T MRA for comparison. The aneurysms were assessed for size, location and morphology.

Results: There were 10 patients with 11 aneurysms ranging in size from 1 mm to 11 mm, with 7 of these being cavernous carotid aneurysms. Catheter angiographic correlation showed good anatomical correlation with the 3D TOF MRA findings in 7 patients. Comparison of the 2 cases with 1.5 T and 3 T MRA showed superior spatial resolution on the 3 T images.

Conclusion: Our experience suggests that with careful visualisation of a 3D TOF MRA at 3 T, even without intravenous contrast, aneurysms down to the size of 1 mm can be detected on the reformatted images with confirmation provided by the source images. This appears to be a suitable non-invasive method of monitoring patients with aneurysms under conservative management.

I017/MP

Neurogenetics and Neuroimaging in Early Alzheimer's Disease.

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Aim: Alzheimer's disease (AD) accounts for more than 40% of dementias in the elderly. Clinical manifestation of AD may be subtle. Early diagnosis is difficult but crucial, to enable early drug intervention and improved prognosis. We applied MR volumetry techniques for the hippocampus, and evaluated for the presence of the apolipoprotein E (ApoE) genotype, a risk factor for AD, to detect the early changes of disease.

Methods: From 103 eligible AD patients, 16 with early AD were recruited. All AD patients were evaluated on the NINCDS-ADRDA criteria, with staging of dementia severity by the Clinical Dementia Rating (CDR) scale. Only those with CDR 0.5–1 (the early stage) and normal unenhanced CT were recruited. 29 normal age-matched controls were also enrolled. All subjects had MR imaging on a 1.5T system, with axial T2W scans to exclude vascular dementia and 3D coronal T1W-SPGR scans for quantitative neuroimaging. Volumetry was performed on BrainVoyager software. All subjects had ApoE typing performed.

Results: Hippocampal volumes in our AD patients were significantly lower than in controls (p < 0.001), despite the clinical early stage of disease by the CDR scale. There were significantly more subjects with positive ApoE amongst the AD group than normals (p = 0.045). Presence of the ApoE genotype was associated with smaller hippocampal volumes in AD subjects.

Conclusion: MR volumetry can detect the subtle changes of hippocampal atrophy in early AD patients. This is associated with presence of the ApoE genotype. Genotypic expression of the ApoE gene results in structural volume reduction on neuroimaging.

I018/MP

Childhood Accidents and Injuries – 1 Year Experience in Children's Emergency

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Aim: To study the details and associated risk factors of childhood accidents and injuries.

Methods: Children (<16 years) with accidents and injuries from 1 February 2002 to 31 January 2003 were studied.

Results: 17032 children were included. 26.8% had accidental injuries and 72.7% without (mean age 7.6 ± 4.6 and 5.2 ± 4.5 years, respectively, $p < 0.001$). 83.6% of the accidents were physical injury (45.2% at home, 17.4% during sports, 12.4% in school, 10.3% in public area and 9.5% in the playground). Other accidents include ENT and GIT foreign body, burns, bites, inhalation injury, near-drowning and poisoning. The commonest diagnoses were scalp and facial injuries (41.0%), limbs fractures (25.1%) and minor injury of the limbs and trunk (25.1%). 60.9% of the playground injuries were fractures, OR 8.7 (95% CI 6.8–11.2, $p < 0.001$) when compared to those at home. Monkey bars were significantly associated with playground fractures (49.5%, χ^2 test, $p < 0.001$). 37.3% of home injuries were head injury, OR 8.3 (95% CI 6.3–11.1, $p < 0.001$) when compared to sports injury. 65.9% of the infants sustained head injury, OR 20.8 (95% CI 13.9–31.3, $p < 0.001$) when compared to teenagers. 94.1% of the infants and 78.4% of the preschoolers had physical injury at home, and 48.9% of the teenagers had sports injury.

Conclusion: Accidents and injuries are common presentations at Children's Emergency. There are certain associated risk factors important in identifying those at risk. The study will help to design measures to prevent such accidents and injuries.

*I019/MP

Depression in Dementia: A Comparative and Validation Study of Four Brief Scales in the Elderly Chinese

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Aim: The study aimed to determine: (i) the diagnostic accuracy of 4 brief depression scales, the Geriatric Depression Scale (GDS), Even Briefer Assessment Scale for Depression (EBAS DEP), Single Question and Cornell Scale for Depression in Dementia (Cornell) in an elderly Chinese population with varying dementia severity, and (ii) which scale had the best diagnostic performance.

Methods: All 4 scales were administered to 88 elderly outpatients with dementia; 66 without and 22 with depression. Receiver Operating Characteristic (ROC) analysis was used to establish the optimal cut-off scores of the GDS, EBAS DEP and Cornell scales. The patients' dementia-severity was categorised into mild and moderate-severe dementia, and the above analysis was repeated in these groups to look at changes in the scales' diagnostic performance as dementia advances.

Results: The best diagnostic scale for detecting depression in dementia was the Cornell scale. Its optimal cut-off score was 6/7 (sensitivity 91.7%, specificity 80.0%) in the mild dementia group and 12/13 (sensitivity 70.0%, specificity 87.0%) in the more advanced dementia group. The optimal cut-off scores of the GDS and EBAS DEP were also higher in the more advanced than the mild dementia group, indicating the increasing difficulty to detect depression with worsening cognitive impairment. The Single Question, being more robust, had lesser changes in its diagnostic parameters in both dementia cohorts: sensitivity 58.3%, specificity 90.0% for mild dementia,

and 60.0% and 84.8%, respectively, for more advanced dementia.

Conclusion: An efficient strategy to diagnose depression in dementia amongst elderly Chinese patients is to first administering the Single Question followed by, when needed, the Cornell scale.

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I020/MP

Findings in the Initial Chest Radiographs Patients with SARS

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Aim: SARS is a new infectious disease caused by a novel coronavirus. Chest radiographs are a crucial part of the initial evaluation of patients that could have SARS. We reviewed the initial chest radiographs from the patients diagnosed as probable SARS.

Methods: The initial chest radiographs from the first consecutive 37 patients diagnosed as probable SARS were reviewed. The films were read independently by 2 radiologists and the findings were tabulated.

Results: Abnormalities were found in 33 patients (89.2%). Unilateral (Right: 46.6%, Left: 10.8%) involvement was more common than bilateral involvement (29.7%). The findings were more common in the lower zone (Lower: 27/33), and in the periphery of the lung. (Peripheral: 32.4%, Central: 18.9%; Both central and peripheral: 37.8%). Ground-glass change was more common (67.6%) than consolidation (16.2%) or a combination of both (5.4%). There was no pleural effusions, masses, cavitation or hilar enlargement.

Conclusion: The chest radiograph findings are not specific and may be subtle. The combination of features in a patient with an appropriate clinical presentation is however suggestive of diagnosis of SARS. These findings are distinct from other common conditions such as pulmonary tuberculosis and classic lobar pneumonia.

I021/MP

Findings in the Initial Chest Radiographs in ICU Patients with SARS

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Aim: Severe acute respiratory syndrome (SARS) is a new infectious disease caused by a novel coronavirus. It progresses to severe acute respiratory distress in a minority of patients. In Tan Tock Seng Hospital, about 20% of patients with SARS required treatment in the Intensive Care Unit (ICU). Chest radiographs are an integral part of monitoring the progress of patients that have SARS. We reviewed the initial chest radiographs obtained after intubation in ICU for patients diagnosed as probable SARS to understand the features of worsening disease.

Methods: The initial chest radiographs from 30 patients diagnosed as probable SARS who were intubated in ICU were reviewed. The diagnosis was based on epidemiological, radiologic and laboratory evidence. The films were read independently by 2 radiologists and the findings were tabulated.

Results: There was a predisposition for symmetric involvement (24/30). Patchy consolidation and ground glass opacities were common findings. No patient demonstrated an interstitial pattern or hilar enlargement. None of the patients had a normal chest radiograph. There was a tendency for the apices and costophrenic angles to be spared. The cardiac silhouette tended to be spared despite severe bilateral involvement.

Conclusion: The chest radiograph findings are not specific and similar to other conditions that cause acute respiratory distress. The findings favor the dependent parts of the lung. This could be related to the underlying pathology of diffuse alveolar damage.

I022/MP

Deep Vein Thrombosis in Patients with SARS

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Aim: Severe acute respiratory syndrome (SARS) is a new infectious disease

caused by a novel coronavirus. It progresses to severe acute respiratory distress in a minority of patients. In Tan Tock Seng Hospital, about 20% of patients with SARS required treatment in the Intensive Care Unit (ICU). As part of protocol for the prophylaxis of deep vein thrombosis, duplex ultrasound of the legs was performed. We describe the prevalence and distribution of deep vein thrombosis in these patients.

Methods: The leg duplex ultrasound scans from 19 patients with probable SARS in ICU were reviewed. The diagnosis was based on epidemiological, radiologic and laboratory evidence. The patients were on a protocol of thromboembolic deterrent stockings and/or low molecular weight heparin. A vascular physician or a radiologist reported the scans.

Results: There was a higher than expected incidence of deep vein thrombosis (7/19) with a predisposition for isolated calf vein thrombosis (6/7). Autopsy in 1 of these patients with calf vein thrombosis showed fragmentary pulmonary thromboembolism.

Conclusion: While immobilisation is a common cause for deep vein thrombosis, the increased incidence and aggressive nature of the disease in some patients despite a protocol for prophylaxis suggest that these ICU patients may require greater intervention.

1023/MP

High-dose Methotrexate (HDMTX) up to 2 g/m² Can be Safely Administered for Childhood Acute Lymphoblastic Leukaemia (ALL) Without Need for Monitoring Serum Levels

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Aim: HDMTX is one of the cornerstones of childhood ALL therapy as it decreases both the risk of CNS and marrow relapses. However HDMTX can be fatal if the patient fails to excrete MTX completely within 72 hours, hence monitoring of serum levels are crucial. Unfortunately, in most developing countries, this is not available and HDMTX is omitted from therapy with resulting poorer outcome. We studied MTX levels at specific time points to determine MTX clearance at 3 standard dosages.

Methods: Renal and liver panels were performed before MTX infusions; MTX levels measured at 24h, 48h and 72h after start of infusion. Folinic rescue was given until MTX level is below target level (48 h <1.0 umol/L; 72 h <0.2 umol/L).

Results: 100 MTX infusions (27 patients) from 04/97-04/03 were enrolled. Mean age was 6 years (range 1-11), normal liver function and mean creatinine before MTX was normal at 39 umol/L (range 21-82). Infusions achieving levels <1.0 umol/L at 48 h or <0.2 at 72h and 120 h were: 100% (n = 12) and 0%, respectively, for 1 g/m²; 90% (n = 44) and 10% (n = 5) for 2 g/m², respectively; 77% (n = 30) and 23% (n = 9) for 5 g/m², respectively. There were no severe adverse complications noted after MTX infusion.

Conclusion: HDMTX of up to 2 g/m² can be given safely without need of serum monitoring provided patient has normal liver and renal functions before infusion. With our results, we recommend that HDMTX up to 2 g/m² can be administered in developing countries without need to monitor serum levels.

1024/MP

Psychiatric Comorbidity and Quality of Life in First Episode Psychosis

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Aim: High rates of psychiatric comorbidity have been reported in patients with psychotic disorders but data is lacking on the quality of life (QOL) in these patients. This study seeks to determine the prevalence rates of psychiatric comorbidity and its clinical correlates in an Asian patient group with first episode psychosis and compare the QOL in these patients with and without psychiatric comorbidity, hypothesising that patients with psychiatric comorbidity would have poorer QOL.

Methods: Within an Early Psychosis Intervention Programme, 131 patients with first episode psychosis were evaluated. The SCID, PANSS, SUMD and GAF were used to assess principal Axis I diagnosis and any other comorbid diagnosis, psychopathology, insight and social/occupational functioning respectively.

Results: Psychiatric comorbidity was present in 34.4 % (n = 45) of the patients. The majority (95.6%) had one comorbid psychiatric diagnosis. Patients with psychiatric comorbidity scored lower on PANSS positive symptom subscale (z = -2.84, p <0.01) but higher on anxiety (z = -3.33, p <0.001) and depression (z = -3.13, p <0.01) items. Patients with psychiatric comorbidity had a greater awareness of their mental illness (z = -3.44, p <0.001) and its social consequences (z = -3.24, p <0.001) but lower ratings on the overall QOL (z = -3.06, p <0.01) as well as in the individual domains (physical, psychological health, social relationships, environment, all p <0.05) compared to patients without any psychiatric comorbidity.

Conclusion: The high prevalence of psychiatric comorbidity with its associated poorer QOL amongst our patients with first episode psychosis warrants attention. The differences in the QOL domains and greater insight may provide potential targets for intervention.

1025/MP

Hippocampal Volume in Patients with First-episode Psychosis

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Aim: To examine the hippocampal volume in minimally treated patients with first-episode psychosis as compared to normal controls.

Methods: Consecutive referrals to the Early Psychosis Intervention Programme were assessed using the Structured Clinical Interview for DSM-IV diagnoses (SCID) and the Positive and Negative Syndrome in Schizophrenia (PANSS). The duration of psychosis (DUP) was determined from the time of onset of first psychotic symptoms to the time of definitive diagnoses. Control subjects with no personal history of psychiatric or neurological illness and no family history of major mental illness, matched for age, gender, and handedness, were recruited from the general population. Both patients and controls underwent MR imaging on a 1.5T scanner with high resolution, fast gradient recalled 3D volumetric scans for structural-anatomic detail. MR data with hippocampal volumetry measurements were analysed off-line using MReg software by an experienced reader.

Results: 32 patients with a mean age of 28.1 (±7.2) years and mean DUP of 12.9 (±18.7) years, as well as 17 controls with mean age of 26.3 (±6.8) years were recruited. Compared to controls, patients had statistically significant smaller mean bilateral (p = 0.028) and right hippocampal (p = 0.004) volumes. Within patient group there was a significant gender difference in bilateral and right hippocampal volumes, i.e. the mean volumes were significantly smaller in females than males. There was no significant correlation between mean hippocampal volumes and patients' age, DUP and PANSS scores.

Conclusion: These findings suggest that reductions in hippocampal volume are present in the early stages of the psychotic illness.

1026/MP

Insight and Attention Deficits in Asian Patients with First-episode Psychosis

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Aim: Poor insight is frequently cited as a contributory factor towards compliance and seeking treatment. The aim of the study is to examine the nature of neurocognitive impairment and explore any correlation with insight in patients with first-episode psychosis.

Methods: Insight and cognitive performance in aspects of executive functioning and attention were assessed in 41 patients. Patients were evaluated within 2 weeks of admission to a State psychiatric hospital. The Wisconsin Card Sorting Test (WCST – computerised version) and the Trail-Making Tests were used to assess for executive functioning. The Continuous Performance Test (CPT-II) and Digit Span (WAIS-III) were used to measure for attention. The NART and Ravens Progressive Matrices (RPM) were also used to assess pre-morbid and current IQ functioning respectively. The

Birchwood Insight Scale served as a self-report measure of insight based on the awareness of illness, need for treatment and attribution of symptoms.

Results: Poor CPT performance was inversely correlated with insight scores. A subscale of the Birchwood insight measuring awareness of illness was also inversely related to deficits on the CPT and Trail-Making Test (Version B). No significant association was found between insight and other cognitive measures, age, gender, or education level.

Conclusion: The present study suggests poor insight is significantly related to attention deficits and some evidence of impairment in executive functioning. Impairment in specific aspects of attention and executive functioning suggest that first-episode psychosis patients may be experiencing difficulty in attending to information. These difficulties may have implications on psychoeducation and compliance in patients with psychosis.

1027/MP

Serum Lipids and Psychopathology in Patients with First-episode Psychosis

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Aim: Several studies have suggested an association of low serum cholesterol levels with depression and increased mortality from violence and suicide. The aim of this study was to examine the association between serum lipid levels and psychopathology in drug-naïve patients with first-episode psychosis.

Methods: All consecutive patients admitted to the Early Psychosis Intervention Programme (EPIP) who consented for the study were recruited. Psychopathology at the time of presentation was assessed using the Positive And Negative Syndrome Scale (PANSS). Blood investigations to measure fasting serum lipid levels were carried out prior to starting the patient on antipsychotic medications. Controlling for age and body mass index (BMI), correlation between serum lipid levels and psychopathology on PANSS was done using Spearman's rank order correlation co-efficient.

Results: 78 patients with the mean age of 29.0 (± 6.0) years participated in the study. 41 (52.6 %) patients were males. There was no significant correlation between serum lipid levels and PANSS total, positive and negative scores. There was a significant positive correlation between PANSS depression subscale and serum triglyceride levels ($p = 0.05$). On individual items on PANSS we found a significant positive correlation between somatic concern ($p = 0.01$) and anxiety ($p = 0.05$) with serum cholesterol levels.

Conclusion: To date, this is the first study to look at the association between psychopathology and serum lipid levels in patients with first-episode psychosis. We did not find an association between psychotic symptoms (positive and negative) and serum lipid levels. Interestingly, the co-morbid symptoms of depression and anxiety were significantly associated with serum cholesterol levels.

1028/MP

Determinants of Duration of Untreated Psychosis and the Pathway to Care in Singapore

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Aim: To elucidate the determinants of Duration of Untreated Psychosis (DUP) in a sample of patients with first-episode psychosis, and to examine the pathways to care in the context of first engagement with psychiatric services.

Methods: The sample comprised patients presenting with first-episode psychosis to the psychiatric services in the year 2000. Combining information from interviews and case records the DUP was estimated and patient's pathway to care was traced. The association between the mean DUP and demographic, clinical and social variables was examined.

Results: Of the 112 patients included in the study, 64 (57%) were men and 48 (43%) were women. The age of onset of psychosis was 38.7 ± 13.7 (mean

$\pm$ SD) years. The DUP ranged from 0.1 to 336 months with a mean $\pm$ SD of 32.6 ± 59.8 months. There was no significant difference in the DUP among those, whose, first contact was the psychiatric services and those who first sought help elsewhere ($p = 0.225$, Mann Whitney *U* test).

Conclusion: The DUP of our patients was longer than that reported in studies done in the West. The attitudes and beliefs of family in the Asian society are likely to be crucial in the pathways to care.

1029/MP

An Emergency Department Response to Severe Acute Respiratory Syndrome – A Prototype Response to Bioterrorism

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Aim: To describe the application of Tan Tock Seng Hospital (TTSH) Emergency Department (ED) disaster response plan to manage the SARS outbreak.

Methods: The ED implemented protection for staff, patients and facility, infection control measures and disaster-response workflow changes. Ministry of Health, Singapore centralised SARS cases in our hospital and our ED became the national screening centre. A screening questionnaire and a set of admission criteria were applied after assessment of clinical features and chest X-ray findings.

Results: For the first 8 weeks of the outbreak, ED attendance was 12,344, of which 8103 (65.6%) were screened for SARS. Among these 8103, 50.2% were men with a mean age of 36.2 years (standard deviation [SD] 15.1) while the mean age for women was 34.8 (SD 14.5) years. Among those screened, 885 (10.9%) were admitted to rule out SARS. Out of 885 admitted, 232 (26.2%) were confirmed to have SARS. Among 7218 persons discharged from ED, there were 28 re-attendances that were admitted and diagnosed to have SARS, giving an under-triage rate of 0.4%. The sensitivity of ED screening and admission for SARS was 89.2% and the specificity was 91.7%. No patient contracted SARS as a result of an ED visit. After full implementation of protective measures, 1 ED nurse with undiagnosed diabetes mellitus was treated for suspect SARS.

Conclusion: Though the SARS outbreak was not a bioterrorism event, the ED disaster response was applied with satisfactory results.

1030/MP

Incremental Value of Serial Closely-spaced Electrocardiograms, Bedside Serum Myoglobin and Quantitative Troponin T and Cardiac Enzymes in the Emergency Department Diagnosis of Acute Coronary Syndromes in Intermediate Risk Patients with a Normal Initial Electrocardiogram

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Aim: We evaluate the usefulness of new bedside methods i.e. closely-spaced electrocardiograms (ECGs), bedside quantitative myoglobin (Cardiac M®) and troponin T (Cardiac T®) and creatine kinase (CK)-MB mass in diagnosing acute coronary syndromes (ACS) in intermediate risk patients with a non-diagnostic initial ECG.

Methods: Patients meeting the entry criteria were recruited. The first blood samples were tested with Cardiac M® and Cardiac T® using the Cardiac Reader® and sent for CK and CK-MB mass laboratory estimations. ECGs were repeated at 15-minute intervals for the first hour. If all these tests were normal, an ECG, and all cardiac markers were repeated at 2 and 4 hours. A discharge diagnosis of acute myocardial infarction (AMI), unstable angina or other diagnoses were made by the in-hospital cardiologists.

Results: A total of 102 patients were analysed; 16 with AMI and 14 with unstable angina. Serial ECGs is the most useful individual early test for diagnosing AMI/ACS. However, 6/13 (46.2%) AMI patients had very subtle ECG changes while 3/16 (18.7%) had normal ECGs and required the cardiac markers for AMI diagnosis. A combination of all 4 tests is better than individual tests for diagnosing AMI and ACS. 100% (95% CI, 79.4 – 100) of the AMI patients were diagnosed in the ED compared to 51.7% (95% CI, 32.5

– 70.6) for ACS. There was 0% in-patient mortality amongst all patients.

Conclusion: A combination of the above 4 tests done up to 4 hours is useful for ruling out AMI but not ACS.

I031/MP

Evolution of an Emergency Department Screening Questionnaire for Severe Acute Respiratory Syndrome (SARS)

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Aim: On 13 March 2003, upon receiving notification of an outbreak of atypical pneumonia, nurses from the study emergency department (ED) started screening patients for this disease that became known as Severe Acute Respiratory Syndrome. This report describes the screening tool that was used, the 3 revisions that were made, and the factors that led to these revisions.

Methods: The ED nurses started with a simple screening tool that was incorporated into triage. The screening tool was later revised into a questionnaire. An outdoor screening station was set up and patients were subsequently screened before triage. After screening, they were assigned to different risk areas, where triage and treatment were rendered. Two further revisions were made to the questionnaire.

Results: In the first eight weeks, 8103 patients were screened. Version One of the screening questionnaire was used to screen 72 patients from 13 to 17 March, Version Two screened 95 patients from 18 to 21 March, Version Three screened 2,918 patients from 22 March to 8 April, and Version Four screened 5,018 patients from 9 April to 7 May. There was a significant ($p < 0.001$) downward trend in the proportion of admissions. Amongst those discharged from ED, 0.4% reattended and was later confirmed to have SARS.

Conclusion: The screening tool underwent 3 major revisions in response to new information. By keeping it relatively simple, user-friendly and regularly updated, nurses were able to screen patients rapidly. Risk categorisation ensured that no cross-infection occurred among patients and that no one contracted SARS in the ED.

I032/MP

Pregnancy Outcomes of Women with Pre-gestational Diabetes Mellitus in Singapore

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Aim: Pregnancy in women with pre-gestational diabetes mellitus (DM) is associated with increased risks to both the mother and her fetus. The aim of our study was to look at pregnancy outcomes in local women with pre-gestational DM, predominantly those with type 2 DM.

Methods: This was a retrospective study based on information obtained from the medical records of women with pre-gestational DM who had been managed during their pregnancy at the antenatal DM clinic at Kangar Kerbau Women's and Children's Hospital from January 2000 to July 2002. Basic biodata and information pertaining to DM were collected. Pregnancy outcomes studied included miscarriage, intrauterine death, delivery complications and mode of delivery. Neonatal complications including macrosomia, congenital malformations and hypoglycaemia were also studied.

Results: There were 151 pregnancies in 134 women. 125 (93.3%) women had type 2 DM, 6 (4.3%) had type 1 DM and 3 (2.2%) had impaired glucose tolerance. The mean gestation at first antenatal visit was 10 weeks with a mean HbA1c of 7.9%. Only 40.9% had a HbA1c of $\leq 7.0\%$. There were 138 (88%) live births, 12 (7.9%) miscarriages and 3 (2%) intrauterine deaths. The rate of caesarean section was high at 53.5%. The mean birth weight was 3261g and the rate of macrosomia amongst singleton pregnancies was 9.3%. Delivery complications included 1 woman with vaginal laceration, 2 with third degree tears and 2 with shoulder dystocia.

Conclusion: A significant proportion of women with pre-gestational DM have suboptimal glycaemic control and present relatively late for antenatal care. The rates of pregnancy complications are significant. Pre-pregnancy planning and education of women with pre-gestational DM needs to be improved to reduce complication rates.

I033/MP

Clinical Usefulness of Endoscopic Ultrasound With/without Fine Needle Aspiration in the Diagnosis and Staging of Pancreatic Carcinoma

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Aim: To show the accuracy and clinical usefulness of endoscopic ultrasound (EUS) with EUS-guided fine needle aspiration (FNA) in early diagnosis and staging of pancreatic cancer not obvious in CT scan abdomen imaging.

Methods: Five male patients were evaluated. Four presented with obstructive jaundice whereas 1 had unexplained loss of weight. Mean age was 66 years (range 40 to 77). All had CT scan abdomen imaging done which did not show any obvious pancreatic tumour. EUS with FNA was done for all cases as indicated. Surgical findings were obtained and compared to EUS findings.

Results: EUS easily detected the pancreatic tumour in all 5 cases. (a) EUS in tumour size assessment: The tumour size detected ranged from 27 mm to 40 mm in diameter. These corresponded fairly accurately with that of surgical findings for the 3 who had surgery. (b) EUS in lymph node assessment: EUS reported 3 out of 5 cases with pathological lymph node involvement. All were histologically confirmed by FNA. The remaining 2 cases of absent pathological lymph nodes were subsequently correctly verified by surgery. (c) EUS in vascular assessment: EUS was accurate in 4 out of 5 cases which reported absence of portal vein or superior mesenteric vein invasion. Surgery could not verify the fifth case. There were no complications at all from the EUS with/without FNA.

Conclusion: This series of cases showed that EUS with/without FNA is very useful to safely diagnose and stage pancreatic head tumour not detectable by CT scanning of the abdomen.

I034/MP

Efficacy of Mirtazapine in Stimulant Associated Insomnia

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Aim: Stimulants are the most commonly prescribed medications for Attention Deficit Hyperactivity Disorder (ADHD). One major side effect of these medications can be an exacerbation or induction of insomnia. In this study, an antidepressant with sedative properties was assessed for effectiveness in treating stimulant-induced insomnia.

Methods: Sixteen patients with early insomnia and ADHD, who were receiving stimulants, were offered 4 to 6 weeks of open treatment with mirtazapine, an antidepressant which is mildly sedating in the lower range of therapeutic doses. (Starting dose: 3.75-7.5 mg ON, final daily dose: 3.75-45 mg/day, mean: 9.4 ± 10.7 SD mg/day). Patients ranged in age from 12 to 47 years. All patients were receiving stable doses of stimulants and met DSM-IV and childhood K-SADS (self or parent report) criteria for ADHD. Sleep patterns were assessed by self-report of average number of hours slept nightly over the past week, at baseline and then during Mirtazapine treatment.

Results: Mirtazapine was well tolerated by fourteen patients. Sleep was improved in all 14 patients (mean hours of sleep: baseline 5.3 ± 1.2 hours; after mirtazapine: 7.9 ± 0.6 hours; $t(13) = 11.7$, $p < 0.0001$).

Conclusion: Mirtazapine significantly improved stimulant associated insomnia in this open, pilot trial.

I035/MP

A Study on Symptoms and Care of Elderly Patients Dying in an Acute Hospital in Singapore

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Aim: To assess the common physical symptoms at the end of life for elderly patients and the management of these patients.

Methods: A retrospective case-control study of symptoms and management

of all elderly patients (>75 years) who died in the year 2001 in Alexandra Hospital using information in their case records.

Results: 189 patients were analysed. The commonest physical symptom at the end of life was difficulty with breathing (58.7%), followed by fever (46.6%), pain (30.7%), and problems with respiratory secretions (14.8%). Other symptoms include vomiting (10.6%), confusion (9.0%) and agitation (5.8%), 72 (38.1%) were comatose. 13 (6.9%) were depressed. 16 (8.5%) of them had smelly wounds and 37 (19.6%) had contractures. Of the group whose death were expected, 52 out of 167 (31.1%) had input from palliative care. Patients who had palliative care input were more likely to be on treatment for pain ($P < 0.05$), and difficulty with breathing ($P < 0.5$).

Conclusion: Elderly patients at the end of life suffer from a number of symptoms which potentially can be controlled with palliative medical treatment. An increase in the treatment of these symptoms is seen with palliative care input.

1036/MP

Nursing Home Falls – A Local Perspective

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Aim: To examine the demography of falls, factors contributory to falls and fall risk factors in residents of a local nursing home.

Methods: A cohort study was carried out on 95 nursing home residents. Each subject's potential fall risk factors was analysed through review of the case records and physical examination. After 18 months, the subjects were reviewed for documented falls, the circumstances surrounding each fall and complications from the fall. The likely factors associated with each fall were categorised where possible. Logistic regression was performed to determine the fall risk factors.

Results: A total of 63 falls contributed by 38 (40%) subjects occurred, giving a fall incidence of 0.5 fall per bed per year. Many subjects were above 75 years old (72.6%), required assistance in basic activities of daily living (71.6%), cognitively impaired (52.6%), diagnosed with mental illness (49.5%), and non-ambulant (43.2%). Thirteen (20.6%) injuries resulted from the falls. Falls associated with both intrinsic and extrinsic factors were identified in 38.1% of falls. The most common intrinsic factors were gait disorders (31.7%) and confusion/difficult behaviour (31.7%). Contributory extrinsic factors include wheelchair/commode (22.2%) and bathroom (15.9%) related incidents. Most falls occurred during ambulation (31.7%) and transfers (17.5%). On multivariate analysis, the only significant risk factors for falls were "need for ambulatory aids" (OR 24.8) and "cognitive impairment" (OR 8.1).

Conclusion: Majority of falls were related to intrinsic factors such as gait disorder and difficult behaviour, and extrinsic factors involving use of wheelchair/commodes and bathroom activities. Thus, nursing home falls reduction protocols should include behavioural measures, enhanced care practices and environmental modifications to be effective.

1037/MP

Novel and Recurring hMLH1 Mutations in Singaporean Families with Hereditary Non-polyposis Colorectal Cancer

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Aim: Hereditary non-polyposis colorectal cancer (HNPCC) is the commonest known form of hereditary colorectal cancer (CRC), and is predominantly due to germline mutations in the mismatch repair genes hMLH1 and hMSH2. Data of the prevalence and spectrum of hMLH1 and hMSH2 mutations in Asians is sparse.

Methods: Singaporean CRC patients diagnosed below age 40, and/or with strong familial cancer clustering suggestive of HNPCC, were tested for tumor microsatellite instability (MSI) and hMLH1 germline mutations.

Results: Forty-seven unrelated high-risk CRC patients were recruited, of which 39 were Chinese, 5 Malay, and 3 Indian. 5/47 fulfilled the Amsterdam I/II criteria, 24/47 were suspected HNPCC kindreds with at least 2 family

members with CRC or HNPCC-like cancer, 5/47 had multiple primary CRC or HNPCC-like cancers, and 13/47 had early-onset CRC. 11/35 patients with tissues available for MSI testing had MSI-high tumors. Five deleterious hMLH1 mutations were identified in 5 Chinese and 2 Indian families, of which 2, the 887T>G (Leucine>termination) and 1851delGAA (deletion of Lysine), in Chinese families, were novel. The other 3 mutations, 380+2T>C (splice defect), 1459C>T (Arginine>termination), and 350C>T (Threonine>Methionine) were previously reported in Caucasians, of which the latter 2 were recurrent in 2 unrelated families each. The data in our Indian families represents the first such data in this ethnic group. In addition, the previously reported 1151T>A (Valine>Aspartate) missense mutation was identified in 3 Chinese families.

Conclusion: Novel and recurring hMLH1 mutations occur in Singaporean HNPCC families. This information is of relevance in formulating molecular diagnostic strategies for HNPCC in Asia.

1038/MP

Effect of ABO Blood Group on von Willebrand Disease Diagnostic Tests in a Chinese Donor Population

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Aim: Diagnosis of von Willebrand disease (vWD) utilizes a combination of quantitative and qualitative tests: ristocetin co-factor assay (vWF:RicoF), and vWF antigen quantification (vWF:Ag). ABO blood group contributes significant to the variation in vWF:Ag levels possibly through differences in vWF glycosylation and clearance. Collagen binding assay (vWF:CBA) is a new test suggested by some to be more sensitive than vWF:RicoF for functional defect of vWF. A study suggests that loss of group A and B antigen affected vWF:RicoF but not vWF:CBA. We investigated the effect of ABO blood group on these tests.

Methods: Citrated blood samples were collected from 55 healthy blood donors (20 group O and 35 non-group O) after informed consent. vWF:CBA and vWF:Ag were performed by ELISA. vWF:RicoF assay was performed by standard method measuring effect of ristocetin on platelet aggregation.

Results: Consistent with previous studies, group O donors had significantly lower vWF:RicoF ($p = 0.0004$), vWF:Ag ($p = 0.0074$) and vWF:CBA ($p < 0.0001$). But in contrast to a previous study, reduction in vWF:CBA is greater than vWF:RicoF in group O donors (48% versus 28%). This leads to higher vWF:Ag/vWF:CBA ratio ($p < 0.0001$) in group O donors whereas vWF:Ag/vWF:RicoF ratio is unaffected ($p = 0.2$). Using our reference range for these tests, 6 O donors would be diagnosed with vWD if only vWF:Ag and vWF:CBA were used.

Conclusion: ABO blood group has significant effect on tests employed in vWD diagnosis. vWF:CBA is affected more than vWF:RicoF by blood group. These variations should be borne in mind when interpreting results of these tests in our population.

1039/MP

Cytotoxicity of Doxorubicin Antiresistant Stealth Liposome on Multidrug Resistance Rat Prostate Cancer Cells

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Aim: To investigate the efficacy of a novel doxorubicin antiresistant stealth liposomes (DARSLs) on multidrug resistance cancer cell.

Methods: Doxorubicin (DOX) and verapamil (VER) were co-encapsulated in liposomes with ammonium sulfate gradient approach. Cytotoxicity of

DARSLs on rat prostate cancer cell line (Mat-LyLu-B2, MLLB2), which expressed multidrug resistance (MDR) gene, was investigated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Real-time uptake of DOX in cells was monitored by confocal microscopy.

Results: The average particle size of DARSLs was 118.6 ± 22.3 nm. Encapsulation efficiencies of DOX and VER in DARSLs were >95% and 70%, respectively. The cytotoxicity of DARSLs on Mat-LyLu-B2 cell line was about 1783 folds higher than that of free DOX. The photographs of confocal microscopy showed the intracellular DOX fluorescence distributed exclusively in cytoplasm after incubation with free DOX (5.5 μ M) for 4h due to expression of multidrug resistance. For DARSLs (DOX 5.5 μ M, VER 5 μ M), DOX fluorescence was mainly in the cytoplasm and slightly in nucleus within 30 min, but, when exposed for more than 150 min, DOX fluorescence was evenly distributed in the nucleus as well as the entire MLLB2 cells.

Conclusion: Our results demonstrated that the formulation of DARSLs can increase DOX accumulation in Mat-LyLu-B2 cell and result in effective inhibition of MDR cell proliferation in vitro.

I040/MP

Flow Cytometric and Histopathological Correlation in the Diagnosis of Lymphoproliferative Disorders

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Aim: To correlate flow cytometry (FCM) and histopathology in lymphoproliferative disorder diagnosis.

Methods: Over 1½ years, 134 consecutive specimens from 110 patients were analysed concurrently by FCM and histopathology/cytology to detect and characterise lymphoproliferative disorders. Specimens submitted for FCM were stained with monoclonal antibodies and their surface and cytoplasmic antigen expressions analysed. Specimens submitted for histopathological analysis were examined by histochemical and/or immunohistochemical stains.

Results: Among 134 specimens, 92 were bone marrow specimens, 23 were lymph node (LN) biopsies, 10 were fine needle aspirates (FNA) of LNs. FCM and histomorphology concurred in 72.4% of specimens (59 negative, 38 positive) regarding the presence or absence of a lymphoproliferative process. FCM and histomorphology were discrepant in 27.6% (37/134). 33 specimens had a lymphoproliferative process by histology but were negative by FCM. 4 specimens had a lymphoproliferative process by FCM but were negative by histology (2 T-lymphoblastic lymphoma, 1 mantle cell lymphoma, 1 Sezary syndrome; all were trephine specimens). All diffuse large cell lymphomas (DLCL)/high-grade lymphomas (29) were diagnosed by histology; 52% (15/29) were detected by FCM. All follicular lymphomas/low-grade lymphomas (15) were diagnosed by histology; FCM detected a higher proportion (80%) compared to DLCLs ($p = 0.002$). Diagnoses solely made by histology included 3 Hodgkin's lymphomas and 2 anaplastic large cell lymphomas. FNAs of LNs yielded 1/6 positive results by FCM. LN biopsies yielded 12/20 positive results by FCM ($p = 0.001$).

Conclusion: Yield in a FCM analysis of a suspected lympho-proliferative disease over histological diagnosis is dependent on the specific diagnosis and type of specimen analysed.

I041/MP

Motivations and Barriers in Attending Cancer Genetics Counselling in Singapore

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Aim: Cancer genetics is an emerging new field. A formal service has been established in Singapore, providing cancer risk assessment, genetic counselling, screening and preventive recommendations. The acceptance and utilisation of such a service has not been studied in Asians.

Methods: We conducted a questionnaire survey in breast cancer patients, cancer-free women, and female health-care professionals (nurses and medical students), asking respondents if they would attend cancer genetics counselling if medically indicated, and to grade the importance of potential motivators and barriers.

Results: 434 women participated, including 164 breast cancer patients, 144 cancer-free women and 125 health-care professionals. The majority had at least "O" level education (67%), used English (64%), was married (56%), and had children (57%). More than 75% indicated willingness to attend genetics counselling if medically warranted, and more than 65% felt they would benefit somewhat to greatly. More than 85% rated 'learning about cancer risk' and 'learning to detect cancer early' as important. Breast cancer women also cited 'helping their family understand cancer risk' as important (94%). Among potential barriers, concerns about 'cost' (57%) and 'inability to comprehend and use the information' (40%) were important. Factors relating to family members (family tells me not to go, not interested, may get upset, may have trouble getting insurance) were not important (less than 30%).

Conclusion: The majority of Singaporean women indicated willingness to attend cancer genetics counselling. Financial cost and inability to comprehend medical information are important barriers, and should be addressed to optimize utilisation of cancer genetics service.

I042/MP

Dorsal Plating for Distal Radius Fracture Using a Low-profile Matrix Plate

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Aim: Dorsal plating in the treatment of displaced unstable distal radius fractures has been associated with complications such as extensor tendon irritation and rupture and dorsal wrist pain. We evaluate the clinical results of a series of patients with dorsal plating using a relatively new implant, the Leibinger Matrix Plate.

Methods: Twenty consecutive patients with displaced unstable distal radius fractures who had undergone fixation using the Leibinger Matrix Plate were reviewed. Only patients with minimum 6 months follow-up were included. The fractures were classified using Frykman, AO and Fernandez classifications. Radiological outcome was assessed using Sarmiento's modification of Lidstrom's scoring system and clinical outcome was assessed using Cooney's modified clinical-scoring system.

Results: The mean age of the patients was 43.2 years (range 24-59 years). There were 13 males and 7 females. 9 of the patients injured their dominant hand, and all fractures occurred after a fall. The results at 6 months according to Sarmiento's modification of Lidstrom's scoring system were 'excellent' in 95% and 'good' in 5%. Post-operatively, the average final radial tilt was 22.2 deg, radial height was 12.7 mm, and palmar tilt was 12.5 deg respectively. Functional end results at 6 months were 'excellent' in 10%, 'good' in 50% and 'fair' in 40%. There were no major complications and no extensor tendon ruptures or irritation.

Conclusion: This study suggests that the Leibinger Matrix Plate is an effective treatment for displaced unstable distal radius fractures, minimizing the complications usually associated with dorsally placed implants.

I043/MP

Can Medical Students Learn Auscultation Without Patient Contact? – Lessons from the 2003 SARS Outbreak

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Aim: The SARS outbreak prevented medical undergraduates from entering teaching hospitals. This study aimed to determine the validity and effectiveness of the electronic stethoscope in teaching auscultation under "no contact" circumstances.

Methods: We used 3M E4000 electronic stethoscopes to record sets of 5 cardiac and 6 respiratory conditions from 11 different patients, using these to teach simulated auscultation to 234 clinical skills foundation course students. Clinical tutors, blinded to diagnoses, independently diagnosed the sets of conditions played on the electronic stethoscope. We measured percentage accuracy to evaluate the validity of these sets. After tutorials, students were tested on a different panel of 5 cardiac auscultations played on the electronic stethoscope. 123 were cluster randomised to a teach cohort which received additional electronic stethoscope teaching, and 111 to a control cohort which did not. We conducted a second test 1 to 12 days after the first with the same auscultations in a different sequence, and mean scores were compared.

Results: Mitral stenosis, mitral regurgitation and aortic stenosis achieved 100% accuracy, normal heart 80% and prosthetic valve 40%. The quality of respiratory auscultations was unacceptable to tutors, thus their use was terminated prematurely. The teach cohort had a higher mean score for correct auscultatory signs during the second test: 3.62 versus 2.94 among controls ($p = 0.001$). There was no difference in this score during the first test.

Conclusion: The electronic stethoscope was a reproducible tool for teaching cardiac but not respiratory auscultations during “no contact” circumstances, producing an incremental learning effect among students with additional use.

1044/MP

Gender Differences in the Endothelial Function of Individuals with Type 2 Diabetes – The Role of Oxidative Stress

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Aim: We hypothesised that endothelial function is impaired in diabetic men compared to women and this impairment is related to oxidative stress.

Methods: We employed 2 dimensional laser Doppler flowmetry to measure the forearm skin perfusion in response to iontophoresis of 1% acetylcholine (ACh, endothelium-dependent) and 1% sodium nitroprusside (NaNP, endothelium-independent) in 100 subjects with uncomplicated type 2 diabetes and correlated the results with plasma ubiquinone (the oxidised subfraction of co-enzyme Q10). Plasma ubiquinone was measured using automated high-performance liquid chromatography (HPLC).

Results: The men ($N = 63$) and women ($N = 37$) were 48 ± 8 (mean $\pm$ SD) and 47 ± 7 years old, respectively. The 2 groups did not differ in terms of clinical and metabolic profile including blood pressure, BMI, waist circumference, fasting blood glucose, HBA1c and lipid profile except HDL was lower in men [1.07 ± 0.26 mmol/L vs 1.37 ± 0.33 mmol/L ($P < 0.01$)]. Ubiquinone was higher in men 38 ± 20 vs. $29 \pm 19 \pm 10.2$ μ mol/L ($P < 0.05$). After adjusting for all the above-mentioned clinical and metabolic profile, the microvascular endothelium dependent and independent vasodilation were reduced in men (1.43 ± 0.79 mV) (Peak mean red blood cell flux after ACh challenge) compared to women (1.71 ± 1.13 mV) ($P < 0.05$). Similarly, endothelium independent vasodilatory response was also reduced in men (1.08 ± 0.67 mV) compared to women (1.50 ± 0.92 mV) ($P < 0.05$). When all the individuals were pooled together, there was a significant negative correlation between plasma ubiquinone concentration and endothelium dependent ($R = -0.26$, $P < 0.05$) and independent ($R = -0.23$, $P < 0.05$) vasodilation.

Conclusion: We conclude that endothelial function is impaired in type 2 diabetic men compared to women. The impaired endothelial function may be related to higher oxidative burden in men.

1045/MP

Persistence of Viruses in the Respiratory Tract in Asthma

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Aim: Respiratory viral infections are common triggers for asthma exacerbations. It is unclear whether viruses can persist in the lower respiratory tract during quiescent asthma. To compare the prevalence and pattern of

common respiratory viruses in the stable, quiescent phase of asthmatic patients with that of healthy individuals.

Methods: Twelve patients with near fatal asthma [NFA], 21 patients with acute asthma [AA] and 52 healthy individuals [C] have been studied. All subjects studied had not been vaccinated against influenza virus. Lower respiratory tract secretions were collected by sputum induction. Specimens underwent RT-PCR for picornavirus; respiratory syncytial virus; influenza A and B; parainfluenza 1 and 3. Fisher's exact test was used to compare prevalence and pattern of respiratory viruses between the 2 groups.

Results: The asthmatic subjects were 41.8 (35-55) [mean range] years in age and the healthy subjects were age 31 (26-35). Viral RT-PCR was positive in 5/12 (42%) NFA, 5/21 (24%) compared with 5/52 (10%) healthy subjects [$p = 0.015$]. Coexistence of viruses [rhinovirus and influenza virus] was found in 5/33 (15%) of asthmatic patients but not in healthy individuals.

Conclusion: These results suggest a tendency for the respiratory tract of asthmatics to be “colonised” by viruses more often than healthy individuals. Further studies will explore this tendency in larger numbers of participants.

1046/MP

The MELD Score is Useful in Determining Optimal Time for Liver Transplantation and Post-transplant Survival for Hepatitis B

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Aim: The aim of our study is to determine if pre-transplant MELD is able to predict post liver transplant survival for Hepatitis B patients.

Methods: This retrospective study included 46 adult patients who underwent primary liver transplantation at the National University Hospital Singapore from January 1996 to December 2002. Pre-transplantation MELD was calculated according to the United Network for Organ Sharing formula. Patients were followed up to the most recent visit or death. Survival analysis was performed using Cox regression for continuous variables and Kaplan Meier for categorical variables

Results: The mean age at transplant was 51 years. The majority of the patients transplanted had Hepatitis B (43%) and were Chinese (72%) and male (80%). 33% had underlying hepatocellular carcinoma and the majority were of blood group O (43%). The median MELD score at transplantation was 17 and the median Child's score was 11(C). There was a correlation between pre-transplant MELD and survival at 1 year post transplant (HR 1.05, 95% CI 0.99-1.12). We found that a pre-transplant MELD score of $\geq$ or equal to 32 had significantly higher mortality at 1 year post transplant compared with those < 32 (HR 9.18, 95% CI = 1.16-72.44). There were no differences in the pre-transplant MELD (OR 1, 95% CI 0.9-1) as well as survival at 1 year for patients with Hepatitis B and those with other aetiologies (HR 0.72, 95% CI 0.22-2.35).

Conclusion: Pre-transplant MELD scores correlate well with survival 1 year after liver transplant and a score of $\geq$ or equal to 32 is associated with a significantly higher mortality at 1 year. There is no difference in survival for patients transplanted for Hepatitis B compared to other etiologies.

1047/MP

The Empirical Antibiotic Treatment of Continuous Ambulatory Peritoneal Dialysis (CAPD)-related Peritonitis – Is it Appropriate?

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Aim: CAPD-related peritonitis is serious and it can be life-threatening if treatment is delayed. Empirical antibiotic at initial presentation is therefore necessary. This is affected by the likely results of the peritoneal fluid culture. To determine the appropriateness of current empirical antibiotic treatment in relation to peritoneal effluent culture result at presentation of CAPD related peritonitis.

Methods: All patients ($n = 45$) with peritonitis episodes in the NUH CAPD programme from 1/1/2002 till 31/12/2002 were studied retrospectively. There were 57 episodes of peritonitis. Microorganism culture, antibiotics used and the treatment outcomes were analysed. All patients received

empirical intra-peritoneal vancomycin and gentamicin at presentation. Antibiotic treatment was subsequently altered according to culture and sensitivity results.

Results: Eleven of 57 episodes (19%) had negative cultures. Forty-one of 57 episodes (72%) were associated with 1 microorganism, 4/57 was associated with 2 organisms and 1/57 episodes was associated with 3 microorganisms. Of the 46 episodes with positive cultures, gram-positive organisms accounted for 40% and gram-negative organisms were responsible for 37%. The remaining were either fungus or negative culture. The 3 commonest gram-positive organisms isolated were coagulase negative Staphylococcus (7 isolates), Streptococcus sp. (6 isolates) and Staphylococcus aureus (3 isolates). The 3 commonest gram-negative organisms isolated were Klebsiella pneumoniae (4 isolates), E. coli (3 isolates) and Acinetobacter sp (2 isolates). Response rate to this antibiotic regimen was 87%.

Conclusion: The current empirical intraperitoneal antibiotic regimen that covers both gram-positive and gram-negative organisms is appropriate in treating more than three-quarter of the CAPD related peritonitis.

1048/MP

Risk Factors for Near-fatal Asthma – A Prospective Study

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Aim: The purpose of the study was to identify factors distinguishing patients with Near Fatal Asthma (NFA) from hospitalised (non near-fatal) acute asthmatic patients (AA) and from community-based asthmatics (CA).

Methods: The design was a multi-centre, prospective study of 81 NFA [mean age, 48.6 (15.6)] who were intubated for acute respiratory failure in the intensive care unit and 311 patients with AA in Singapore. An interviewer administered questionnaire and knowledge assessment were conducted 24-48 hours after admission. A random sample of community-based asthmatics [n = 46] were recruited for comparison with hospital controls.

Results: Age distribution was similar between AA, NFA and CA. There were more women than men in the NFA and AA groups [58% and 66%] compared with the CA [36%] [p = 0.001]. There was no difference in the history of allergies, age of onset or years of treatment for asthma. Compared with CA, patients with NFA and AA had previously received more asthma education, inhaler and peak flow training [p = 0.001], had better inhaler technique [p = 0.003], had better knowledge scores for asthma medication and acute attack self-management, but had poorer scores for therapeutic compliance, self-efficacy and patient-doctor relationship [p = 0.001]. NFA patients were more likely than AA to have a previous ICU admission and recent poor asthma control [p = 0.001].

Conclusion: Poor control of asthma and reduced therapeutic compliance are associated with NFA despite good asthma education.

1049/MP

Vasomotor Reflexes of the Radial, Ulnar and Brachial Artery – A Study of Normal Range, Repeatability and Influencing Factors in Healthy Humans

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Aim: Vasomotor reflexes (VMRs) are a useful test of small unmyelinated (sympathetic) nerve fibers. VMRs are usually measured from skin vessels but can be elicited in many vascular territories. Since skin VMRs are particularly susceptible to emotional and environmental stimuli and represent only a relatively small portion of the vascular innervation, it is useful to establish VMR of other vascular territories. This study investigates the range of VMR in vessels supplying skeletal muscles of the upper limb, their repeatability and influencing factors (age, gender, weight, height, blood pressure).

Methods: Bilateral vasomotor reflexes of the radial, ulnar and brachial arteries were recorded using Ultrasound Doppler. Testing was repeated at 3

separate times. VMR was determined by measuring the pre and post inspiratory gasp difference in diastolic blood flow and expressed as a percentage.

Results: 64 healthy adults (age 21-78) participated. Ranges (mean $\pm$ SD) for radial VMR (right to left respectively) were 78% ($\pm$ 12.9)/ 85% ($\pm$ 12.5), ulnar 86% ($\pm$ 11.6) / 88% ($\pm$ 13.0); brachial 68% ($\pm$ 14.5) / 72% ($\pm$ 16.3). Radial, ulnar, brachial VMR correlation was good (P <0.01). Repeatability using intraclass correlation was 0.5390-0.6782. Ulnar VMR was best (P <0.01). Diastolic BP affected ulnar VMR (P <0.05). Age, gender, weight, height had no effect at all locations (P for all >0.1).

Conclusion: Mean VMR for all vessels was above 68% and highest for the ulnar artery which is best suited for studies. Repeatability was good at all sites. Apart from diastolic BP, age, gender, weight and height did not significantly affect vasomotor reflexes.

1050/MP

Preventable Causes of Hospital Admissions Due to Epilepsy: A Prospective Study in Singapore

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Aim: Chronic conditions such as epilepsy exert considerable strain on health services resources in part due to repeated hospital admissions for seizures. To prevent unnecessary admissions, it is important to first understand the reasons for admission. This study examines the preventable causes for admission to hospital for patients suffering from epilepsy in Singapore.

Methods: Over a 6-month period, patients with a generalised seizure due to epilepsy and on antiepileptic medication admitted to the Neurology inpatient service of 2 hospitals (National University Hospital and Alexandra Hospital) were prospectively studied for the cause of hospital admission. Using a structured interview and additional tests, the following seizure precipitants were sought: compliance, serum antiepileptic levels, sleep deficit, alcohol, dyselektrolytemia and infections.

Results: Of the 42 enrolled patients (25 male, 17 female), 28 suffered from idiopathic, 9 from scar and 5 from a variety of causes of epilepsy. In 33, at least 1 seizure precipitant could be identified. Of the total of 35 identified precipitants, 27 subjects had 1, 7 subjects had 2 and 1 subject had 3 precipitants. 20 patients were non-compliant, 28 had subtherapeutic serum antiepileptic levels, 7 had sleep deprivation and in 1 each, alcohol abuse, low sodium and infection was identified. 9 had no identifiable precipitant.

Conclusion: Our prospective study examining potentially preventable causes for seizures leading to hospital admission for patients with epilepsy identified that the majority of patients have potentially preventable causes of their seizures and thus hospital admission. Targeted education will be an important public health strategy.

1051/MP

Skin Nerve Fibre Density and Neurophysiological Parameters in Neuropathy

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Aim: The method of counting skin intraepidermal nerve fiber density (IENFD) for diagnosing sensory small nerve fiber neuropathy is established. But, its role in the overall assessment of sensory polyneuropathy remains unclear. This study investigates the relation of hand IENFD with neurophysiological parameters of large and small nerve fibre function of upper and lower limbs in predominantly sensory neuropathies.

Methods: In patients with predominantly sensory polyneuropathy, sensory motor nerve conduction of ulnar, peroneal and sural nerves as well as palm, sole sympathetic skin response (SSR) were performed. Skin biopsy for IENFD was taken from the mid-hypothenar region, nerve staining used PGP 9.5 antibodies.

Results: Tests were performed in 33 patients (13 male, 20 female; mean age 57 years; range 20-88) with neuropathy. SSR was done in 26 (16 male, 10 female). Neuropathy was due to diabetes mellitus (type 2) in 11, 9 cryptogenic,

4 diabetes and renal failure, 3 renal failure, 3 diabetes and alcohol, 2 CIDP and both 1 with diabetes mellitus (type 1) and vitamin B12 deficiency. IENFD nerve counts/mm ranged from 0.0-3.76 (mean laboratory control value = 4.72; SD 1.6). IENFD correlated significantly with ulnar (motor $p = 0.008$; sensory $p = 0.0135$), sural ($p = 0.008$) conduction velocity and hand SSR ($p = 0.012$) but not peroneal nerve ($p = 0.072$) or sole SSR parameters ($p = 0.134$).

Conclusion: In sensorimotor polyneuropathy of different aetiologies, IENFD and neurophysiological parameters of large and small nerve function correlate well if test sites are matched. This supports the usefulness of IENFD as a parameter of sensory polyneuropathy in general.

I052/MP

Angiotensin Receptor Blockers are Superior to Angiotensinogen Converting Enzyme Inhibitors in Preserving Renal Function in Patients with Nephropathy from Type 2 Diabetes Mellitus

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Aim: Selective angiotensin receptor I blockers (ARB) have been demonstrated to reduce proteinuria and preserve renal function in diabetic nephropathy (DN) due to type 2 diabetes (T2DM). The evidence for angiotensinogen converting enzyme inhibitors (ACE-I) in this area is not as clear. We hypothesize that ACE-I and ARB are equally efficacious in terms of renal protection in DN due to T2DM.

Methods: We analysed the 6-months follow-up data of 53 patients with DN from T2DM, comparing the change in creatinine clearance (CCT) over 6 months (calculated from Cockcroft-Gault equation) with ACE-I and/or ARB use.

Results: The percentages of patients on no ACE-I/ARB, ACE-I alone, ARB alone and combination therapy with ACE-I and ARB were 10%, 32%, 25% and 33%, respectively. The changes in calculated CCT over 6 months in the 4 groups were -4.03 ± 1.33 ml/min, -3.64 ± 6.68 ml/min, 1.56 ± 6.60 ml/min and 2.20 ± 6.40 ml/min, respectively. The differences in CCT change between patients on ACE-I alone compared to patients on either ARB alone or combination therapy were statistically significant ($p < 0.05$). There was no statistically significant difference in CCT change between patients on ARB alone or on combination therapy. Baseline renal function, blood pressure, HbA1c and lipid profile were similar among the 4 groups. The improvement in CCT with ARB and combination therapy was independent of change in blood pressure. There was no statistically significant difference in serum potassium levels in all 4 groups.

Conclusion: ARB and combination therapy appeared to be superior to ACE-I in preserving renal function in patients with DN from T2DM.

I053/MP

Treatment Outcome of Continuous Ambulatory Peritoneal Dialysis (CAPD)-related Peritonitis at National University Hospital (NUH)

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Aim: Management of CAPD-related peritonitis involves consideration of appropriate use of antibiotics and timely removal of Tenckhoff catheter if treatment fails. To review treatment outcome of CAPD related peritonitis in relation to culture results.

Methods: Thirty-two patients with CAPD-related peritonitis presented from 1/1/2002 till 31/12/2002 were included. Data on microorganism culture, antibiotics used were collected and analysed in relation to treatment outcome. Treatment failure defined as failure of significant clinical improvement after initiating therapy for 72 hours that required Tenckhoff catheter removal.

Results: There were 55 episodes of CAPD-related peritonitis between 1/1/2002 and 31/12/2002. Forty-four episodes were due to bacteria, while 11 episodes were culture negative. All patients received intra-peritoneal vancomycin and gentamicin at presentation. Twelve episodes required the use of either ciprofloxacin or ceftazidime. Fifty episodes (90.9%) resolved after treatment while 5 episodes (9.1%) had treatment failure. For treatment

failure group, culture results revealed *Pseudomonas aeruginosa* (2), *Staphylococcus aureus* (1) and culture negative (2). Percentage of treatment failure by different culture results were 6.8 and 18.2 for bacterial and culture negative peritonitis respectively. Interestingly, about one-fifth of culture negative peritonitis group required Tenckhoff catheter removal. There may be an increase trend of catheter removal in the culture negative group, however, this has to be further elucidated.

Conclusion: Our empirical antibiotic regimen has a very high success rate for treatment of CAPD peritonitis. However, effective treatment has to be balanced with culture and sensitivity results and prudent use of antibiotics, which needs to be tailored accordingly.

I054/MP

Detection and Cloning of a luxS-encoded Autoinducer from Toxigenic *Clostridium difficile*

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Aim: Bacteria regulate gene expression in a cell-density dependent manner in response to extracellular signaling molecules by a process called quorum sensing. These signaling molecules, called autoinducers (AIs) play important roles such as bioluminescence, sporulation, virulence, conjugation and biofilm formation. Recently, a novel autoinducer called autoinducer-2 (AI-2) was discovered. AI-2 production is luxS-dependent and was hypothesised to be a "universal" signaling molecule for interspecies communication. Does *C. difficile* possess a LuxS-based quorum sensing system?

Methods: Cell-free supernatant from *C. difficile* CCUG 19126 was tested for AI-2 activity in the AI-2 bioassay which uses *V. harveyi* BB170 as the reporter strain. Degenerate oligonucleotide primers were designed to amplify a portion of luxS from *C. difficile* genome by polymerase chain reaction (PCR). The PCR product was cloned and used as a probe in Southern blot hybridisation for subgenomic library construction. About 10,000 library colonies were screen via colony hybridisation. The putative luxS homologue was identified and cloned into luxS mutant *E. coli* DH5 α for complementation studies.

Results: Toxigenic *C. difficile* CCUG 19126 produces functional AI-2 in a growth phase dependent manner. Maximal activity was observed during early to mid-exponential phase of growth. Southern blot hybridisation indicated that the gene involved in the synthesis of this signal exist as a single copy in the genome. A luxS homologue from *C. difficile* was identified, cloned and showed to complement AI-2 production in the luxS mutant *E. coli* DH5 α . Amino acid alignment analysis indicated high homology with LuxS from various bacteria.

Conclusion: A LuxS-based quorum sensing system exists in *C. difficile* and further studies would be important to elucidate the roles AI-2 may play in this pathogen.

I055/MP

A Study of the Birth Weight Distribution in Healthy-term Newborns Among Different Races

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Aim: To define the range of birth weight of healthy babies for different races at increasing gestational age after 37 weeks.

Methods: Birth weight, race, gestation, gender and other demographic data were prospectively obtained from the neonatal notes and entered into the NHG electronic database from 1 July 2002. During the period from 1 July 2002 to 31 May 2003, there were 2184 healthy term babies born during this period. Of these, complete data from 1102 babies were available for analysis. Babies included were those whose gestation were >37 weeks. There were 542 Chinese babies, 343 Malay babies and 94 Indian babies. The frequency distribution of birth weights by race and by gestation was analysed.

Results: The mean birth weights for Chinese, Malays and Indians were 3219 g, 3129 g, and 3099 g, respectively. The mean birth weight for Indian babies appeared to peak at an earlier gestation of 40 weeks compared to Chinese and Malay babies which peaked at 41 weeks. An average female baby weighed 3120g, 122g lighter than an average male baby. The percentages of small for

gestational age (SGA) (<2.5 kg) babies for Chinese, Malay and Indians were 3.3%, 6.7% and 6.5%, respectively. The 10th and 90th percentiles in the entire study population were 2650 g and 3739 g, respectively.

Conclusion: This study provides current data on birth weights of healthy term babies from different races in Singapore. This helps doctors to better evaluate newborns in the local population and provide optimum care for them.

1056/MP

Neurological Diseases Among 15,000 Older Singaporeans – A Community-based Prevalence Survey

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Aim: This paper describes the self-reported prevalence of dementia, epilepsy, parkinsonism and stroke among Singapore adults aged > 50 years.

Methods: This is a cross-sectional community-based survey of neurological diseases 25,000 Singaporeans aged >50 years living in central Singapore. Cross-sectional disproportionate stratified random sampling by ethnic group was used. Trained interviewers conducted face-to-face interviews with subjects using a standardised questionnaire and validated screening tools. Subjects were also asked if they had ever been told by a doctor to have had dementia, epilepsy, parkinsonism or stroke. Data was entered into an Excel file, and analysed using SPSS for Windows.

Results: 14906 subjects participated, response rate 66.6% (2698 ineligible). Female to male ratio was 1.2:1. 41.2% were aged 50-59 years, 34.2% 60-69 years, 18.4% 70-79 years, 6.5% 80 years. Self-reported prevalence of stroke was 3.9%, dementia 0.6%, parkinsonism 0.3% and epilepsy 0.2%. Stroke prevalence was higher among females (4.5% vs 3.3%, $p < 0.001$), older subjects (trend $p < 0.001$), and those with less formal education ($p < 0.001$). Dementia prevalence was similar for females and males, higher among older subjects (trend $p < 0.001$), and those with less formal education ($p < 0.001$). Parkinsonism prevalence was similar for females and males and across educational levels, more prevalent among older subjects ($p < 0.001$). Epilepsy prevalence was similar for females and males and across educational levels, more prevalent among older subjects ($p < 0.02$). No inter-racial differences in prevalence rates were found.

Conclusion: The prevalence of self-reported stroke was 3.9%, dementia 0.6%, parkinsonism 0.3% and epilepsy 0.2%. Stroke was more prevalent among males. Stroke, parkinsonism, epilepsy and dementia were more prevalent among older subjects. Funding: National Medical Research Council, National Healthcare Group.

1057/MP

Clinical and Electrophysiological Correlation in Patients with Suspected Carpal Tunnel Syndrome – NNI Experience in 2002

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Aim: 1) Clinical profile of patients presenting with carpal tunnel syndrome (CTS). 2) Correlation of clinical parameters with electrophysiological tests.

Methods: A retrospective study of 120 patients with clinical suspicion of CTS was performed. The parameters included were age, gender, side affected, duration and type of symptoms, associated conditions, treatment and outcome. Electrophysiological studies were performed and the results were graded into mild, moderate and severe CTS.

Results: 120 patients referred here met the electrodiagnostic criteria for CTS. The mean age was 53.1 years. Most patients were female (87.5%) and Chinese (84.2%). Sensory symptoms like numbness and paresthesias were the predominant symptoms (90.8%); only 14.2% patients showed abnormal findings of weakness (10%) and wasting (4.2%). 27 (22.7%) was documented to have diabetes mellitus and 6 (5%) had hypothyroidism. On electrophysiologic testing, 47 patients (39.2%) had unilateral and 73 (60.8%) had bilateral hand involvement, curiously only 48 of these 73 patients had

bilateral hand symptoms. 71 patients (59.2%) had the involvement of right hand. Overall, 41 patients (34.2%) had mild, 57 (47.5%) had moderate and 22 (18.3%) had severe CTS. 15 patients with moderate and severe CTS patients underwent surgical treatment with 12 showing improvement of symptoms post operatively.

Conclusion: Our study shows that: 1) Chinese women account for majority of the patients; 2) Most patients present in the 5th decade; 3) Asymptomatic bilateral hand involvement is seen commonly in the electrophysiologic tests despite unilateral hand symptoms; 4) Right hand involvement is more common; 5) Most patients present with sensory symptoms. Abnormal physical findings are less common; and 6) Surgical treatment results in resolution or improvement of symptoms in those with moderate and severe CTS.

1058/MP

Identification of Mutations in X-linked Charcot-Marie-Tooth Patients in Singapore

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Aim: Charcot-Marie-Tooth disease comprises a group of clinically and genetically heterogeneous inherited motor and sensory peripheral neuropathies. The X-linked dominant form of Charcot-Marie-Tooth disease (CMTX) is due to mutations in the connexin 32 (CX-32) gene located on the X-chromosome. It is the second most common form of hereditary motor and sensory neuropathy after PMP22 duplication and shows both demyelinating and axonal features. Although the clinical, pathophysiological and genetic basis of CMTX has been intensely studied, mutations in the CX-32 gene have not been systematically screened for in Singapore.

Methods: We screened 15 patients for mutations in the CX-32 gene by direct sequencing. In addition to patients suspected to have CMTX by their clinical or electrophysiologic findings, patients suspected to have CMT1 and who had a negative test for PMP22 duplication were also included.

Results: Three unrelated patients showed distinct missense mutations in their CX-32 gene. These are an A→G base change at codon 34 (Met34Val), an A→G substitution at codon 73 (Met73Val), and a G→A alteration at codon 159 (Gly159Asp).

Conclusion: These mutations have been previously reported in the literature but they are the first to be described in the Singapore population. These findings suggest that CMTX is not uncommon in Singapore. Coupled with the clinical heterogeneity of CMTX patients, it would be advisable to include CX-32 screening in patients with inherited peripheral neuropathies.

1059/MP

A Homozygous Mutation in UDP-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene (GNE) in a Patient with Distal Myopathy with Rimmed Vacuoles (Nonaka Myopathy)

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Aim: The study aims to identify mutations in UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) gene, a causative gene for distal myopathy with rimmed vacuoles (DMRV), also known as Nonaka myopathy. DMRV causes distal weakness and wasting of the lower limbs, occurs with young adult onset and shows autosomal recessive inheritance. The GNE gene maps to the chromosome 9p12-p11.

Methods: cDNA was isolated from blood samples of a patient with the clinical and histopathological phenotype of DMRV. All 13 GNE exons were amplified from the patient's cDNA by PCR with specific sets of primers and sequenced using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Results: Direct sequencing of the GNE coding region revealed a homozygous

missense substitution G→A, converting valine to methionine (V696M) in exon 12. This missense mutation (V696M) was previously reported (Eisenberg et al, *Nature Genetics*, 2000) in a compound heterozygous form in a patient with autosomal recessive inclusion body myopathy, another form of hereditary myopathy which is allelic with DMRV.

Conclusion: This is the first report of a patient with DMRV showing this homozygous mutation in exon 12 of GNE. It is also the first mutation identified in a patient with DMRV in Singapore.

1060/MP

The Blood Oxygen Level Dependent (BOLD) Response to the Temporal Distribution of Interictal Epileptiform Discharges

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Aim: The BOLD response to the distribution of interictal epileptiform discharges (IEDs) has not been systematically characterised. Stimuli timing in conventional fMRI is crucial and affects efficiency, which relates inversely to the variance of the parameter estimates. We investigated the BOLD response to IED distribution using the concepts of detection (DE) and estimation efficiency (EE), hypothesising that efficiencies and BOLD response levels would positively correlate.

Methods: Data acquired from 5 idiopathic generalised epilepsy patients with 3Hz spike-and-wave IEDs (simultaneous EEG-fMRI: 1.5T scanner, referential EEG). DE and EE were calculated using Friston et al's (1999) formula. BOLD responses were modelled, based on the different aims of DE and EE, using different basis functions. Event onsets and durations were considered individual parameter estimates. Maximum F-ratios were correlated with corresponding efficiency values.

Results: Number of EEG events: 6-138; event interval range: 0.4-627.9 s; event duration range: 0.3-15.1 s. Significant BOLD activations ($p < 0.05$) were revealed in 22 of the 24 analyses. For models featuring event onsets alone, no significant correlations between efficiencies and F-ratios were found [DE: $r_s = 0.657$ ($p = 0.156$); EE: $r_s = 0.60$ ($p = 0.208$)]. There was also no significant correlation for the models including event durations [DE: $r_s = 0.086$ ($p = 0.872$); EE: $r_s = 0.086$ ($p = 0.872$)].

Conclusion: The temporal distribution of EEG events did not appear to be the main determinant of significant BOLD responses. All events and durations entered as parameters were assumed equally meaningful, however later events within a cluster could reflect propagation effects. Including event durations in the model yielded smaller lower F-ratios and correlation coefficients, possibly reflecting the inferiority of the models.

1061/MP

Cerebral Venous Thrombosis: Subtle Signs on Neuroimaging

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Aim: Cerebral venous thrombosis (CVT) is a potentially fatal condition. The clinical presentation is non-specific, and without a high index of suspicion, the diagnosis is often missed. We reviewed neuroimaging findings in patients to determine if there were subtle signs of CVT that might be helpful in patients in whom the diagnosis is not suspected.

Methods: An imaging database of final diagnosis of CVT was created from picture archive and communications system in the National Neuroscience Institute. Patients with confirmed diagnosis of CVT and at least 1 imaging study performed in our institution were included. Retrospective analysis of the CT, MR and angiography studies was carried out. Computed tomography, with or without intravenous contrast, was performed in all patients. MR study comprised at least T1- and T2-weighted and in some cases post-enhancement sequences and time of flight MR venography.

Results: There were 19 patients with dural sinus thrombosis (7 male, 12 female; age 15 to 81 years). All diagnoses were confirmed on either MRV or

angiography. There were 5 cases with parenchyma hematomas, 2 of which also had subarachnoid haemorrhage (SAH). In all patients who had contrast-enhanced CT studies, the "empty delta" sign was detected. There were prominent venous collaterals present in 12 patients manifested as dural or leptomeningeal enhancement in 3 patients.

Conclusion: CVT can present with the empty delta sign or isolated subarachnoid haemorrhage on CT. On MR imaging, abnormal combined leptomeningeal and dural enhancement are findings that support the diagnosis of CVT.

1062/MP

Hypoxia Inducible Factor-1 α and Expression of Vascular Endothelial Growth Factor and its Receptors in Cerebral Arteriovenous Malformations.

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Aim: In previous studies, vascular endothelial growth factor (VEGF) and its tyrosine kinase family of receptors (VEGFR) have been implicated in vascular angiogenesis and remodelling in cerebral arteriovenous malformations (CAVM). In this study we investigate the role of hypoxia inducible factor-1 α (HIF-1 α) in CAVMs and its relationship with VEGF and VEGFR.

Methods: We studied 26 patients who underwent surgery for CAVM. Patient demographics, clinical presentation and AVM characteristics were obtained from our prospectively maintained database. The specimens were studied for HIF-1 α , VEGF, Flt-1, Flk-1 and Flt-4.

Results: The mean age was 34.08 ± 14.18 years. Twenty-one patients presented with intracerebral haemorrhage. VEGF, Flt-1 and Flt-4 were expressed in all specimens. Flk-1 was expressed in 15 of 26 patients. HIF-1 α was expressed in 15 of 26 patients. HIF-1 α expression was not associated with patient age, gender, nidus size, haemorrhage, timing of surgery and GCS at surgery ($p > 0.05$). However it was significantly associated with VEGF, Flt-1 and Flk-1 expression ($p < 0.05$).

Conclusion: The expression of HIF-1 α is related to VEGF and VEGFR expression, suggesting a possible role for its induction and role in maintaining angiogenesis and vascular remodelling.

1063/MP

Exogenous Spermine Reduces Infarct Volume in the Rat MCA Occlusion Model

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Aim: The role of polyamines in the pathophysiology of cerebral ischemia has not been well elucidated. Spermine augments the excitotoxicity mediated by the N-methyl-D-aspartate (NMDA) receptor because this receptor is activated at micromolar spermine concentrations. However at higher concentrations, spermine could be neuroprotective because it blocks the NMDA receptor and voltage-activated calcium channels. In this study, we aim to investigate the role of spermine in focal ischemia using MRI diffusion imaging techniques.

Methods: Adult Sprague-Dawley rats (300-330 g) were anesthetised with a clinical research cocktail. The left middle cerebral artery was occluded with intraluminal suture. IV infusion of 10 mg/kg spermine (or saline for the control group) was administered via the femoral vein 30 minutes post occlusion. 24 hours later an MRI was performed with the Bruker 2T MRI machine with a custom made rat coil. Coronal images 1.2 mm thick with 3 mm gaps were acquired with TE 52.6 msec, TM 29.2 msec, big delta 50 msec and b values of 300 s/cm² and 30,000 s/cm².

Results: The mean volume of cytotoxic edema as reflected by abnormal apparent diffusion coefficient in the spermine group was 170.25mm³ compared with 472.12 mm³ in controls (2 tailed t-test $p = 0.016$).

Conclusion: Exogenous spermine administered 30 minutes after MCA occlusion ameliorates infarct/ injury volume. Further study is required to ascertain if this may translate in the human setting to significant neuroprotection from acute brain injury.

1064/MP**Intravenous Immunoglobulin Treatment for Toxic Epidermal Necrolysis: Are There Ocular Benefits?**

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Aim: To evaluate acute ocular complications of toxic epidermal necrolysis (TEN) following treatment with human intravenous immunoglobulin (IVIG) compared to a historical cohort not treated with IVIG.

Methods: Consecutive patients with TEN as defined by Bastuji-Garin et al. presenting between 1 July 2001 and 30 June 2002 were treated with high dose IVIG (2 g/kg body weight over 2 days). Patients' records were retrospectively reviewed for their demographic characteristics, causative drug, treatment, ocular involvement (if any, as assessed by an ophthalmologist) and its severity. A historical cohort was obtained from patients coded with a diagnosis of TEN (ICD Code 695.1) between 1 July 1995 and 30 June 2001.

Results: Nine (90%) of 10 patients treated with IVIG had ocular involvement. Phenytoin was the implicated drug in 3 (37.5%) patients. IVIG was initiated within 6.8 ± 3.9 days (range 3 to 14) of onset of TEN. Of the 9 patients, 1 died of septic shock. Of the 8 survivors, ocular complications were mild in 2 (25%) (lid oedema or mild conjunctival injection), moderate in 5 (62.5%) (conjunctival injection, pseudomembrane, symblepharon, corneal epithelial loss which healed with conservative treatment) and severe in 1 (12.5%) (non-healing epithelial defect requiring surgery, visual loss). The historical cohort comprised 10 (55.6%) of 18 patients with TEN who had acute ocular involvement. Three patients died. Ocular involvement was mild in 6 (60.0%) cases and moderate in 4 (40.0%) with no severe cases.

Conclusion: IVIG did not appear to reduce the rate or severity of visually significant ocular complications.

1065/MP**Hollenhorst Plaques in the Retinal Arterioles – A Case Series**

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Aim: The significance of embolic cholesterol crystals, also known as Hollenhorst plaques, in the retina of patients with occlusive cerebrovascular disease is well established. However, the relationship between Hollenhorst plaques with the status of the carotid artery is not a direct one. We report a case series of 7 patients with cholesterol emboli in the retina and correlate the findings to their ultrasound carotid findings, medical risk factors as well as lipid profile.

Methods: All patients seen in the eye clinic with positive findings of Hollenhorst plaques between year 2000 to 2001 were identified. Clinical risk factors were recorded and all patients underwent carotid duplex doppler study and a lipid profile assessment.

Results: A total of 7 patients were identified. Six patients had unilateral embolus and 1 had bilateral emboli. All patients were male with diabetes mellitus. Six patients had concomitant hypertension. Calcified plaques were noted in the carotid arteries of all subjects and were associated with varying degrees of stenosis on both sides. All patients had dyslipidaemia.

Conclusion: Patients with Hollenhorst plaques should routinely be screened for risk factors such as diabetes, dyslipidemia and hypertension. Varying degrees of carotid stenosis can occur in patients with Hollenhorst plaques and carotid ultrasound remains an important non-invasive tool in assessing its severity.

1066/MP**Steroid-resistant Focal Segmental Glomerulosclerosis in Children – Predictors of Non-response to Therapy**

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Aim: Focal segmental glomerulosclerosis (FSGS) is a common cause of primary nephrotic syndrome in childhood and has been associated with a poor response to corticosteroids and progression to end-stage renal disease.

Cyclophosphamide and cyclosporine, which have potential long-term complications, are often used as second-line treatment in these patients. This study aimed at identifying the parameters that could be predictive of poor outcome to non-corticosteroid immunosuppressives and progression to chronic renal failure in children with steroid-resistant FSGS.

Methods: The results from 210 consecutive renal biopsies at the Department of Paediatrics, National University Hospital, were examined. The medical records of patients with steroid-resistant nephrotic syndrome due to FSGS, were analysed. Factors studied, using both univariate and multivariate logistic regression analyses, included gender, race, age at diagnosis, hypertension, haematuria and serum creatinine at initial presentation, tubular atrophy, and initial or late steroid-resistance.

Results: 23 children with steroid-resistant FSGS were included in the study. 3/14 (21%) children responded to cyclophosphamide. 8/17 (47.1%) children responded to cyclosporine. 11/23 (47.8%) children progressed to renal failure. Haematuria at first presentation ($p < 0.03$) and tubular atrophy ($p < 0.05$) were associated with non-response to immunosuppressives. Multivariate analysis showed that only haematuria was predictive of non-response to non-corticosteroid immunosuppressives ($p < 0.03$, OR 16.0, 95% CI 1.7-176.5). Non-response to immunosuppressives ($p < 0.02$, OR 24.0, 95% CI 2.1-279.6) was predictive of progression to renal failure.

Conclusion: By identifying patients with steroid-resistant FSGS who are less likely to respond to immunosuppressive therapy and therefore at risk of progression to renal failure, potential side effects of these drugs can be avoided.

1067/MP**Reference Values for Lung Function in Healthy Asian Preschool Children by the Impulse Oscillation Technique**

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Aim: Asthma is the most common chronic childhood disorder with peak prevalence at preschool age. Preschool children are unable to perform standard lung function tests like spirometry. Novel techniques like impulse oscillometry are required. Reference values for preschool lung function are established in Caucasian children but not previously studied in Asian children. The objectives were to determine reference values for lung function in Asian preschool children (aged 2-6 years old).

Methods: Healthy preschool children (without a history of asthma, chronic cough or wheeze; and with a normal respiratory examination) were identified from kindergarten. Dual measurements of respiratory resistance (Rrs) at 5, 10, 15, 20, 25, and 35 Hz; respiratory reactance at 5 Hz (Xrs5) and total respiratory impedance at 5 Hz (Zrs5) were performed by impulse oscillometry.

Results: Measurements were successful in 36 (94.7%) of 38 children with a mean age of 4.2 years (range 2.3 to 6.6 years). None of the indices of respiratory function differed between boys and girls. The regression equation for Rrs5 in kPa L-1 s with height in cm was $Rrs5 = -0.021 \text{ height} + 3.2585$. The regression equation for Xrs5 in kPa L-1 s with height in cm was $Xrs5 = 0.008 \text{ height} - 1.1687$. The regression equations for Rrs5 and Xrs5 in this study of healthy Asian preschool children compare favourably to the equations obtained previously from healthy Caucasian preschool children.

Conclusion: The reference values for lung function in healthy Asian preschool children established using the impulse oscillation technique can be applied in future clinical and epidemiological studies in Asian children.

1068/MP**The Combination of Multiplex RT-PCR and DNA Index is Superior and Can Effectively Replace Conventional Karyotyping in Stratifying Childhood Acute Lymphoblastic Leukaemia (ALL)**

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Aim: Accurate risk stratification in childhood ALL is crucial to optimize the risk-benefit ratio of treatment. This is currently achieved by grouping patients into specific genetic subgroups with differing prognoses. Unfortunately, conventional karyotyping is both laborious and only successful in ~40-70% of patients. We evaluated the combination of a multiplex RT-PCR panel and DNA index (multiplex RT-PCR/DI) against current gold standard karyotyping

in risk stratification in B-lineage ALL.

Methods: We utilised a multiplex RT-PCR assay which detects 29 chromosomal translocations/aberrations in 7 parallel reactions including more than 80 mRNA breakpoint or splice variants. The freshly obtained mononuclear cells for flow cytometry were fixed and stained with propidium iodide. DNA Index (DI) was calculated as the ratio of peak channel number of aneuploid and diploid G1 phase cells. Conventional karyotyping of bone marrow samples was performed in the Karyotyping Laboratory with standard techniques.

Results: Diagnostic RNA samples were available in 59 out of 72 patients. The combination of multiplex RT-PCR/DI successfully assigned 60% (43/72 patients): 21% (15) TEL-AML1, 6% (4) E2A-PBX1, 4% (3) BCR-ABL, 3% (2) MLL-AF4, 1% (1) TLS-ERG, hyperdiploid 24% (17) and hypodiploid 1% (1). Karyotyping successfully assigned 36% (26/72) patients. Specifically, karyotyping failed to detect all TEL-AML1 ($n = 15$) patients who have excellent prognoses, 2 patients with MLL-AF4 as well as 1 patient each with E2A-PBX1 and TLS-ERG that have adverse prognoses.

Conclusion: The combination of multiplex RT-PCR/DNA index is superior to conventional karyotyping in risk stratification of childhood ALL (60% vs 36%). It is most useful for detection of important oncogene fusions arising from submicroscopic chromosomal translocations, eg. TEL-AML1, MLL rearrangements that are missed by karyotyping.

1069/MP

MTT Assay is a Valuable New Marker for Determination of Prednisolone Sensitivity in Childhood Acute Lymphoblastic Leukaemia (ALL): An *In vitro* Evaluation Model Using Leukaemia Cells Lines

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Aim: Cellular resistance to treatment is the most important cause of relapse in childhood ALL and is biggest problem that prevents further improvement beyond 75% long-term cure currently attained. Accurate and reproducible measurements of cellular drug resistance may provide prognostically significant information that is useful for improved risk-group stratification. Poor prednisolone response at day 8 is a powerful determinant of outcome but is only applicable to 10% of patients. Our objective was to determine if *in vitro* sensitivity to prednisolone (PRD) using methyl-thiazol-tetrazolium (MTT) assay is feasible, reproducible and applicable to all patients. We first seek to explore this in 3 established leukaemia cell lines.

Methods: The sensitivity of 3 ALL cell lines were measured using colorimetric MTT assay at 6 different concentrations of prednisolone over 4 days.

Results: Interestingly, REH cell line was found to be most resistant, followed by 697 (E2A-PBX1) then SUP-B15 (BCR-ABL) to prednisolone. Prednisolone concentration of 0.4 $\mu\text{g/ml}$ was the optimal concentration that distinguishes the cell lines from sensitive to resistant. The reproducibility of the assay was studied in multiple duplicates at this concentration and the average viability at day 4 post prednisolone was 88% (REH – resistant) versus 45% (697 – intermediate) and 30% (SUP-B15 – sensitive) with good reproducibility. Interestingly, this is different from conventional wisdom that REH (TEL-AML1) is more sensitive than SUP-B15 (BCR-ABL). But this may be due to these cell lines were derived from relapsed patients and may have unpredictable sensitivity.

Conclusion: MTT assay can be a valuable *in vitro* assay for prednisolone sensitivity in childhood ALL and may be useful for gene expression profiling studies.

1070/MP

Estimation of Pulmonary Vascular Resistance in Congenital Ventricular Septal Defects: A Simplified Approach

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Aim: Quantification of the magnitude of left to right shunt (Qp/Qs) and determination of pulmonary vascular resistance (PVR) remain a major indication for cardiac catheterisation for patients with congenital left to right

shunt. However, computation involves multi-step mathematical calculations, different methods of estimation of mixed venous oxygen saturation (MVO₂) and different assumptions of oxygen consumption (VO₂), resulting frequently in manual calculation errors. The purpose of this study are: (1) to simplify computation of all the relevant haemodynamic indices using computer-assisted calculations, with input of relevant parameters by user, and (2) to propose a simplified equation to calculate PVR, using only Qp/Qs and assumed cardiac index.

Methods: All computer-assisted computations are manually checked and verified to be correct. To compare PVR obtained using conventional methods and that using the simplified equation, clinical data of 207 consecutive patients with isolated ventricular septal defects (VSD) were entered into an Excel file. The purpose-written computer programme in C-language computed all the derived indices, including MVO₂, Qp/Qs, VO₂ and PVR.

Results: The correlation coefficients between PVR by simplified equation and conventional methods (using LaFarge and Kappagodda formulae) were 0.946 and 0.957, $p < 0.0001$, respectively. There was no systemic bias comparing the conventional and simplified approach by Bland and Altman analysis. The positive predictive and negative predictive values of high PVR by the simplified approach vary from 0.75 to 1.00.

Conclusion: The simplified approach allows error free computation of all haemodynamic parameters and a quick reliable estimate of PVR in patients with congenital ventricular septal defects.

1071/MP

Molecular Involvement of JAG1 Gene in Patients with Alagille Syndrome (AGS)

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Aim: The JAG1 gene codes for a ligand in the Notch signalling pathway which plays a critical role in cell fate determination and differentiation. Abnormalities in this gene have been associated with Alagille syndrome, a multisystem developmental disorder involving the liver, heart, skeleton, eye and facial features. This study aims to investigate the molecular basis of 5 patients with clinical features suggestive of Alagille syndrome (AGS).

Methods: Clinical assessment was carried out based on histologic findings from liver biopsy, and involvement of cardiac (most commonly pulmonary vascular defects), skeletal, ocular, facial and pancreatic abnormalities. Molecular analysis was performed by DNA sequence analysis of all 26 exons and flanking regions of the JAG1 gene.

Results: Five patients were identified based on histologic findings of paucity of intrahepatic bile ducts, and association of at least 3 of the 5 major clinical findings: cholestasis (80% of cases), cardiac abnormalities (100%), skeletal abnormalities (80%), ocular abnormalities (50%) and characteristic facial phenotype (50%). Molecular characterisation of the JAG1 gene revealed the presence of truncating mutations which are predicted to lead to premature truncation in translation of normal JAG1 protein. All the nonsense codons were generated from frameshift insertions or deletions of single or multiple nucleotides.

Conclusion: The molecular basis of 5 patients with features of AGS was determined and the involvement of JAG1 was confirmed from the truncating mutations identified. Molecular analysis of JAG1 gene is useful for confirmation of clinical cases of AGS especially due to the high phenotypic variability of this disease which range from subtle sub-clinical findings to life-threatening liver or heart disease.

1072/MP

Hand Injuries in Children

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Aim: To understand the epidemiology, sites and mechanism of hand injuries that occur in children. To consider safety measures that can be implemented with the above-mentioned knowledge.

Methods: A retrospective study was carried out with a specifically designed questionnaire. Data is taken from attendees from the National University

Hospital Children's Emergency over the period of February 2002 to January 2003. Using the database available, information on the diagnosis, mechanism of injury and x-ray findings reported by the radiologists were extracted.

Results: 4568 trauma cases were seen over this period of time, of which 414 (9.1%) patients had hand injuries (250 were male and 164 were female). The mean age was 8.8 years with a range of 2 months to 16 years. The right hand was injured in 207 patients (50.0%), the left hand in 199 patients (48.1%) and both hands in 8 patients (1.9%). Laceration was the most common reason for presentation with a total of 121 (26%) patients, Seventy-six (18.6%) patients had fractures while 85 (20.5%) had minor sprains and strains. "Jamming/crushing" was the commonest reason (24.4%) for isolated finger injuries.

Conclusion: Although hand injuries are not life threatening, they are sources of pain for children and of psychological trauma for children and caregivers. They are usually due to jamming in doors, resulting in cuts of soft tissue, nail or bony injuries, including amputation. Both children and adults should be educated about the mechanism and causation, emphasizing that injuries can be prevented by safety measures.

1073/MP

Molecular Pharmacological Mechanisms of PX3.102 – An Active Component of a Chinese Medicinal Herbal Decoction For Autoimmune Disease Treatment

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Aim: CMX-13, a bioactive component of a Chinese herbal decoction, was found to be effective for the treatment of lupus nephritis as well as prevention of rat lung allograft rejection. Recently PX3.102, its active compound has been purified and it was shown to suppress PHA stimulated PBMC proliferation in a dose dependent manner. This study further investigated its molecular pharmacological mechanisms using cDNA microarray assay.

Methods: PBMCs were isolated from 2 normal healthy subjects by Ficoll-Hypaque. PBMCs were stimulated by PHA and treated with PX3.102 (dissolved in DMSO) at 5 ng/ml and 10 ng/ml, respectively at 37°C. PHA-stimulated PBMCs treated with DMSO served as controls. Proliferation assay was performed simultaneously to evaluate PBMC responsiveness. After 24 hours of incubation, cells were washed with PBS and harvested for cDNA microarray analysis (Clontech). Genes with ratio more than 1.8 were considered as upregulated, whereas those with ratio less than 0.56 were considered downregulated.

Results: 8 genes were found upregulated by PX3.102 in PBMCs, including interleukin 6, interleukin 1, placental growth factor. In contrast, 22 genes (metallothionein 2A and 1G, cathepsin B and D for example) were downregulated. These genes involved multiple pathways including transcriptional activation, antigen degradation, immunoglobulin production as well as apoptosis, which was in agreement with our previous functional study.

Conclusion: These findings suggest that PX3.102 may work through multiple pathways in PBMCs including apoptosis pathway. Further studies are ongoing to confirm its role in these pathways, and their interactions will be determined.

1074/MP

The Effect of CETP Taq1B Polymorphism on Plasma Lipid Levels Among Singaporean Neonates

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Aim: The Taq1B polymorphism, a silent base change at nucleotide 277 in intron1 of the cholesteryl ester transfer protein (CETP) gene, has been reported to be associated with plasma HDL-cholesterol levels. The association has not been investigated in neonates

Methods: Allele frequency determination and plasma lipid profiles were determined in; Chinese (n = 435), Malay (n = 308) and Indian (n = 223) neonates who had no history of mixed heritage in the preceding 3 generations. Deviation of genotypes from Hardy Weinberg proportions were tested by the chi-square analysis. Significance of differences in allele frequencies between

groups were determined by the z-test while differences in lipid profile levels between genotypes were tested by analysis of covariance.

Results: The B2 allele frequency was significantly higher (P < 0.02) among the Indians (0.43) when compared to Malays (0.37). Chinese male homozygotes for the B2 allele had higher levels of apoA-1 (P < 0.0005) and lower levels of triglycerides (P < 0.009) when compared to subjects with B1B2 and B1B1 alleles. In both sexes in all ethnic groups there were no statistically significant differences among the genotype groups in the plasma levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, apoB levels and lipoprotein(a) levels.

Conclusion: The study showed significant influence of the Taq1B polymorphism on HDL related plasma apoA-1 and triglyceride levels at birth among the Chinese subjects only. However, no significant association with HDL-cholesterol levels were observed.

1075/MP

The Changing Prevalence of Asthma and Allergies in Singapore – Suggestion of a Plateau in Trends

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Aim: The prevalence of asthma in Singapore has been increasing over the last few decades. More recent reports have suggested a plateau in this trend in many countries around the world. The aim of this study was to evaluate the current asthma prevalence amongst Singapore schoolchildren and its changing trends.

Methods: A cross-sectional study using the standardised ISAAC questionnaire-based protocol was carried out in 2001 and the findings compared with those of previous studies in Singapore (1997, 1987 and 1967).

Results: 9308 children were surveyed using instruments identical to a previous study in 1994. Over the last 7 years, the symptoms of current wheeze (wheeze in the last 12 months) showed a decreasing trend [1994: 12.0% vs 2001: 11.0%]. This trend was not in the same direction in both age groups, decreasing in the 6-7 year age group (16.6% to 10.2%) but increasing in the 12-15 year age group (9.9% to 11.9%).

Conclusion: The prevalence of current wheeze, a surrogate measure of asthma prevalence, has not increased substantially in Singapore schoolchildren in recent years and there is a suggestion of a plateau in its upward trend.

1076/MP

Acute Drug-Induced Dystonias in the Children's Emergency

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Aim: To describe the important clinical features of 5 patients that presented with acute drug-induced dystonias to the Children's Emergency over a ten-month period, and to highlight the risks of anti-emetic-induced dystonias in children.

Methods: Medical records of the 5 patients were reviewed and a literature review was conducted.

Results: We report 5 boys aged 6.8 to 15.3 years (median age 14.8 years), of which 4 were 12.7 to 15.3 years old. All had no previous dystonias. Among them, 3 had reactions to metoclopramide, 1 to prochlorperazine and 1 to haloperidol. Metoclopramide and prochlorperazine were prescribed for acute gastroenteritis and haloperidol for severe tics. Three of them had dosages above recommended ranges. The dystonias occurred 21 to 72 hours (median 26 hours) after starting the medications and involved extraocular, buccolingual and/or cervical muscles. All responded readily to intravenous benzotropine and/or diazepam. One patient had a recurrence. Literature review shows that in patients less than 30 years old, the incidence of acute dystonia-dyskinesias is 1:81 for metoclopramide and 1:464 for prochlorperazine. Males, age group 10 to 19 years and patients with previous dystonias have higher risks. The commonest implicated drugs in children are anti-emetics. Dystonias are more likely if the dosages exceed recommended doses and/or are repeated. Common differential diagnoses include meningitis, seizures and tetany.

Conclusion: Judicious use of anti-emetics in children is crucial, especially if risk factors are present. Practitioners should be familiar with the recommended dosages, risks, clinical features and management of acute dystonias.

1077/MP

Cell Block Immunocytochemistry Helps in the Differentiation Between Adenocarcinoma and Mesothelial Lesions

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Aim: The differentiation of adenocarcinomas and mesotheliomas is of prognostic, therapeutic and medico-legal importance. With pleural fluid being an initial early sample often obtained from such patients, we seek to validate the usefulness of cell block immunocytochemistry as a diagnostic aid and to propose a simple panel for the diagnostic work-up.

Methods: Cell blocks were prepared from pleural fluid of 5 cases of adenocarcinomas, 4 cases of mesotheliomas and 3 cases with reactive mesothelial cells (RM), all of which had diagnoses established clinicopathologically. The panel of immunostains evaluated comprised Ber-EP4, CEA, B72.3, Leu-M1, EMA, Calretinin and Thrombomodulin.

Results: All cases of mesotheliomas and RM were negative for Ber-EP4 and CEA while all except 1 case of adenocarcinoma were positive for these stains; there was generally stronger and more extensive staining for Ber-EP4 in adenocarcinoma. B72.3 and Leu-M1 appeared to be slightly less discriminatory. All cases of mesotheliomas and RM were positive for Calretinin, displaying the characteristic fried egg cytological appearance, while adenocarcinomas were negative/weakly staining. Thrombomodulin staining was generally selective for mesothelioma, except for a single case of adenocarcinoma with aberrant staining. EMA was positive in all cases, although a differential staining pattern was sometimes discernable (cytoplasmic for adenocarcinoma cells; cell surface accentuation for mesothelial cells).

Conclusion: Cell blocks provide good cytology material for the immunocytochemical analysis of suspected adenocarcinoma /mesothelial lesions. They should always be prepared whenever possible. An initial immunocytochemistry panel comprising Ber-EP4, CEA, Calretinin and Thrombomodulin is suggested as an indispensable aid in the cytodiagnosis of these lesions.

1078/MP

RAS Oncogene Mutations in Intestinal and Diffuse Type Gastric Carcinomas in Singapore

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Aim: Gastric cancer is the third most common cancer in Singapore. We had previously studied the 2 main subtypes of gastric cancers in Singapore and found that there is a differential expression for a variety of antigens, including the RAS oncoprotein. Subsequent studies from Japan, Korea and Florence (Italy) have also provided conflicting data. The incidence of RAS mutations also appear to vary and these regional variations could be due to whether the studies were conducted in high incidence (eg. Florence and Japan) or low incidence regions.

Methods: The methods used were 3-fold. Genomic extraction of DNA from paraffin-embedded tissue slides, PCR amplification of RAS exons and Denaturing Gradient Gel Electrophoresis (DGGE) of PCR -amplified exons for point mutation analysis.

Results: A total of approximately 70 patients were selected for this project. Exons 1 and 2 of the Ki-ras2 and N-ras genes were analysed. No mutation were detected for any of the exons for either of the genes with the exception of a mutation on exon 1 of the Ki-Ras2 gene for 1 of the patients. This mutation occurred consistency on DGGE gels and demonstrated a unique DGGE band profile consisting of 4 bands. DNA sequencing confirmed the presence of this mutation occurring on codon 12 of the exon in which a G had been changed to a T on the first base of the triplet. A functional mutation was apparent possibly altering glycine to cysteine on the protein.

Conclusion: RAS mutations are an infrequent occurrence in gastric carcinogenesis. Our rates are similar to those in countries with a low incidence.

1079/MP

Venous Thromboembolism in Intensive Care Patients with Severe Acute Respiratory Syndrome

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Aim: Severe acute respiratory syndrome (SARS) is a flu-like coronavirus infection with progression to pneumonia. A proportion of patients develop respiratory failure requiring intensive care (ICU) management. We report a high incidence of venous thromboembolism (VTE) in SARS patients in ICU and our evolving management strategies.

Methods: A retrospective review of all probable SARS patients admitted to ICU from March 15 to May 12, 2003 was conducted. Deep vein thrombosis (DVT) was diagnosed by compression Doppler ultrasound (CUS) or at post-mortem. Pulmonary embolism (PE) was identified at post-mortem or diagnosed clinically. Low-molecular-weight heparin (LMWH) thromboprophylaxis evolved from none to high dose anti-Xa level guided dosing.

Results: 18 VTE events were identified in 31.1% of this cohort (14/45). Mean age of patients with VTE was 46.8 years. 7 patients had DVT alone, 3 had PE alone and 4 patients had both. Other than the first 2 patients who had PE and DVT diagnosed at post-mortem, the rest of these patients had LMWH thromboprophylaxis. Despite increasing dose of LMWH, VTE were still encountered. No established pre-ICU VTE risk factors were identified. Mortality for the VTE group presently stands at 57.1%.

Conclusion: A significant proportion of SARS patients in ICU developed VTE despite aggressive thromboprophylaxis. This leads to considerable mortality. Further studies are needed to elucidate pathogenesis of VTE in SARS to guide future management.

1080/MP

Tubularised Incised Plate (TIP) Urethroplasty: Expanded Use in Primary and Secondary Hypospadias Repair

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Aim: We evaluated our experience using the TIP technique for primary distal, proximal hypospadias as well as re-operated cases.

Methods: We retrospectively reviewed the records of 56 boys (mean age of 3.02 years) operated by 4 consultant surgeons in our institution between December 1998 and December 2002. 53 patients had primary repairs while 3 were secondary reconstruction following failed urethroplasty with alternate techniques. The meatal positions were coronal in 15 (27%), distal in 19 (34%), mid-shaft in 19 (34%) and proximal in 3 (5%). TIP urethroplasty consisted of tubularised widened hinge urethral plate with 6/0 absorbable PDS suture. The neourethra was further reinforced with 2 layers of de-epithelialised dartos tissue. Glanuloplasty and meatoplasty were completed with interrupted sutures. All patients were stented postoperatively with the duration of urinary diversion range from 3 to 14 days (mean 8.5 days).

Results: Mean follow-up was 14.3 months (range 3-45 months). 53 (95%) patients had the meatus in the glanular position with vertical orientation. Complications of urethrocutaneous fistula occurred in 6 (11%) of patients while meatal stenosis was found in 5 (9%) patients. No urethral stricture or recurrent chordee has been encountered thus far.

Conclusion: The TIP urethroplasty achieved satisfactory functional and cosmetic outcome in both primary and complicated hypospadias repair in our centre.

1081/MP

Highly Specific and Sensitive Molecular Diagnosis of Fragile X Syndrome Using a Triple Ms-PCR Strategy

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Aim: Fragile X syndrome is the most common inherited form of mental

retardation, and is caused by hyperexpansion and hypermethylation of a polymorphic CGG repeat in the 5' untranslated region of the FMR1 gene, in >95% of cases. Normal (NL) alleles contain 6-55 CGGs, while full mutation (FM) alleles have >200 CGGs, are silent, and are derived from at-risk but functional premutation (PM) alleles (55-200 CGGs). More than a decade after its gene identification, Southern blot analysis remains the only reliable method to detect all possible FMR1 alleles in both sexes, due to the refractiveness of this locus to PCR. We wished to devise a simpler, more rapid alternative to Southern blot analysis for the molecular diagnosis of fragile X syndrome, but with equivalent specificity and sensitivity.

Methods: DNA samples were subjected to sodium bisulfite conversion, and a triple PCR protocol (nonmet-PCR, met-PCR, and mTP-PCR) was utilised to unambiguously genotype NL, PM, and FM males and females by simple agarose gel analysis.

Results: Using this protocol, we correctly determined the FMR1 (CGG)n genotype of 44 male and 45 female samples that have been genotyped previously by Southern blot analysis. Including the pre-PCR sodium bisulfite genomic modification step, results were obtained within 2 days, compared with 1 to 2 weeks for Southern blot analysis.

Conclusion: The triple ms-PCR assay is the most comprehensive PCR-based system available for the diagnostic evaluation of fragile X syndrome in both males and females, and represents a significant advance over Southern blot analysis.

1082/MP

Pharmacokinetics of Vancomycin in Asian Patients Undergoing Intermittent Hemodiafiltration

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Aim: This study characterised the pharmacokinetics of vancomycin in Asian patients undergoing intermittent hemodiafiltration (HDF).

Methods: Postdilutional HDF was performed in ESRD patients who had suspected or documented infections requiring vancomycin therapy. 1.0 gm vancomycin was infused over 60 minutes within 30 minutes of commencement of HDF. Serum samples were simultaneously sampled, in duplicates, before and after the dialyzer at 1 h, 2 h, and at the end of HDF. Three other samples were collected at the start, 1 h post-HDF and just before the next scheduled HDF session. Serum vancomycin was measured using fluorescence polarisation immunoassay. The pharmacokinetics of vancomycin was determined using a 1-compartment model. Student's t test was used for statistical analysis.

Results: Seven patients (mean age $\pm$ SD; 57.6 $\pm$ 9.52) were studied. Serum vancomycin concentrations decreased 53% from 25.0 $\pm$ 6.3 mg/L to 10.3 $\pm$ 2.5 mg/L at the end of HDF. Correcting for rebound, the actual concentration at the end of HDF was 11.6 $\pm$ 2.7 mg/L. The calculated volume of distribution was 0.41 $\pm$ 0.08 L/kg. The intra- and interdialytic half-lives were 2.95 $\pm$ 0.68 hrs and 128.9 $\pm$ 68.8 hrs respectively. The serum vancomycin concentration before the next HDF session, 48 hours later, was 9.13 $\pm$ 2.86 mg/L.

Conclusion: A 3-hour HDF treatment session significantly reduces vancomycin levels in patients with renal failure. This is in contrast to conventional hemodialysis treatment in which there is little clearance of vancomycin. Assuming a thrice weekly intermittent HDF treatment prescription similar to our study conditions, vancomycin should be dosed at each HDF treatment. The patient's residual renal function should be taken into consideration to further optimise dosing.

1083/MP

Low Concentrations of Resveratrol Inhibits Apoptosis-induced by Known Chemotherapeutic Agents in Human Leukemia Cells: A Word of Caution and an Insight into an Environment Non-permissive for Apoptotic Execution

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Aim: Recently, we have highlighted the critical role of a permissive intracellular milieu in the efficient execution of the apoptotic signal. Consistent with that, we showed that apoptosis induced by exogenous or drug-induced intracellular

hydrogen peroxide (H₂O₂) was mediated by a decrease in intracellular superoxide (O₂⁻) concentration and cytosolic acidification. Contrarily, a slight increase in intracellular O₂⁻ concentration inhibits apoptotic signaling.

Methods: MTT and propidium iodide staining for cell death, fluorometric analysis of caspases, mitochondrial membrane potential and hydrogen peroxide production.

Results: Here we show that low concentrations of the chemopreventive agent resveratrol (4-8 μ M), trigger a substantial increase in intracellular O₂⁻ production in HL60 human leukemia cells. Pre-incubation of tumor cells to resveratrol blocks apoptosis induced by exogenous H₂O₂ or by exposure to anti-cancer drug merocil that kills via intracellular H₂O₂ production. Cells pre-incubated with resveratrol neither undergo a decrease in intracellular O₂⁻ nor a drop in cytosolic pH in response to H₂O₂ or merocil. Consequently, there is a decrease in caspase activation, and a block in H₂O₂-induced mitochondrial engagement. Interestingly, we have observed that this phenomenon of resveratrol induced inhibition is common to modern anti-cancer drugs such as vincristine, paclitaxel and daunorubicin.

Conclusion: These results suggest that unlike its pro-apoptotic activity at higher concentrations, resveratrol at relatively low concentrations can act as a pro-oxidant and by doing so inhibit the execution of the apoptotic signal. Our data could have tremendous implications for the use of resveratrol in combination chemotherapy regimens, particularly with compounds that signal through intracellular H₂O₂ production.

1084/MP

A Study of a Programme to Improve Vestibular Function in Patients with Multiple Sclerosis who are Community Ambulant – A Pilot Study

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Aim: To determine the efficacy of a tailored, supervised programme to improve vestibular function in patients with multiple sclerosis (MS). It also aims to investigate the correlation between the balance and mobility measures and a self-perceived disability measure.

Methods: Seventeen subjects with MS were randomly allocated to the experimental and control groups. The experimental group participated in a customised and supervised intervention in the form of workstation model twice a week for 6 weeks. The control group received a "sham" home exercise programme for 6 weeks. The unipedal stance, sharpened Romberg, modified Clinical Test for the Sensory Interaction on Balance (mCTSIB), and the functional reach (FR) tests measured balance. Functional mobility measures included the time up and go test and the dynamic gait index. The dynamic visual acuity test and dizziness handicap inventory evaluated dizziness and gaze stability respectively. The Multiple Sclerosis Impact Scale measured self-perceived measure of disability of MS.

Results: The experimental group demonstrated a significant improvement in FR compared to the control group post-intervention ($P = 0.046$, $P < 0.05$). The improvement in FR was not significant after 1 month follow up ($P = 0.309$, $P > 0.05$). The group did not demonstrate significant improvement post-intervention and after 1 month with the other dependent variables ($P > 0.05$). The sharpened Romberg and dizziness handicap inventory had moderate correlation with most of the other balance and functional mobility measures.

Conclusion: The vestibular function of MS subjects was not significantly improved following a 6-week tailored intervention. However, frequently practised tasks such as reaching significantly improved.

1085/MP

Do Severe Acute Respiratory Syndrome (SARS) Precautionary Measures Affect Patient's Satisfaction with Doctor-patient Interaction?

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Aim: Measures have been implemented by Singapore's hospitals to contain Severe Acute Respiratory Syndrome (SARS), including personal protective

equipment, week-on week-off rosters, mandatory temperature checks and visitor restrictions. We assessed the impact of these measures on patient satisfaction.

Methods: As part of the department's quality improvement programme, out-patients seen at National University Hospital Radiotherapy Centre during the SARS outbreak completed an anonymous, previously validated questionnaire assessing satisfaction with doctor-patient interaction. Items also assessed patient perception of SARS measures.

Results: Of 149 patients, 97% had heard of SARS and 92% believed SARS precautions necessary. 54% believed contracting SARS was possible despite the measures. Patients were satisfied with doctors wearing masks (97%), temperature checks (97%), patients wearing masks (96%) and visitor restrictions (88%). 24% believed SARS measures had adversely affected doctor-patient interaction. 94% of patients were satisfied with their doctor-patient interaction. Issues patients were most satisfied about included understanding their treatment plan (100%), doctor being honest (97%), being understood (96%), explanation of treatment (95%) and addressing patients' concerns (94%). However, 61% believed they could have been told more about how to care for their illness, 59% felt that the visit could have been better, 58% believed the doctor could show more interest, and 21% felt rushed during consultation. Patients who were less satisfied with SARS measures were significantly less satisfied with doctor-patient interaction ($p = 0.0001$).

Conclusion: Overall, patients were satisfied with doctor-patient interaction despite implementation of SARS prevention measures. We identified specific areas of interaction for targeting by the departmental quality improvement programme.

1086/MP

Gender Differences in a 1-year Cohort of Patients with First-Episode Psychosis in Singapore

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Aim: To examine gender differences in age of onset of illness, duration of untreated psychosis (DUP), demographic parameters and response to treatment in a 1-year cohort of patients with first-episode psychosis.

Methods: The sample comprised 94 Asian patients presenting with first-episode psychosis in the year 2001. Detailed demographic information was collected, and patients were assessed regularly on the Positive and Negative Syndrome Scale (PANSS), Global Assessment of Functioning Scale (GAF) and WHO-Quality of Life Scale (QOL-BREF).

Results: The group comprised 54 (57%) men and 40 (43%) women. The men were significantly younger ($p = 0.03$), single ($p < 0.005$) and had received more years of education ($p = 0.05$). There were no significant differences in the DUP among men and women. There were no significant differences in the GAF, PANSS [although poor impulse control was assessed to be significantly higher in men ($p = 0.009$)] or QOL-BREF scores at baseline. Men had significantly more readmissions ($p = 0.03$) in the 1-year follow-up period, however response to treatment did not vary within the groups.

Conclusion: Gender differences in patients with first-episode psychosis were similar to those of chronic patients. Rehospitalisation rates also followed a similar pattern with men having significantly more hospitalisation and longer stays.

1087/MP

The Unmet Need for Effective Therapy in Ankylosing Spondylitis

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Aim: We evaluated how many of our patients would meet the proposed ASAS criteria for initiation of biologic therapy in ankylosing spondylitis (AS) (BASDAI > 40), and aimed to establish robustness of the criteria, evaluating quality of life and work disability

Methods: 325 consecutive clinic attendees with a diagnosis of AS were

mailed a postal questionnaire, of whom 228 (70%) returned it. We assessed disease activity (BASDAI), function (BASFI, HAQ, LDQ), quality of life (ASQoL), and work instability (WIS). Student t-tests were used for comparison between groups.

Results: The mean age of these patients was 47.4 ± 12.4 years and 25% were female. Mean BASDAI was 48.9 ± 23.6 , with 65% of patients had BASDAI > 40 . The ASAS cut-off point was proven to be robust, with highly significant differences between the 2 groups in functional ability (BASFI [$p < 0.001$] and HAQ [$p < 0.001$]), quality of life (ASQoL [$p < 0.001$]), and predictably pain ($p < 0.001$). BASDAI > 40 was also found in 44% of those who were working and associated with a significantly higher risk of work instability ($p < 0.001$). Overall, 20% of them had lost their job because of AS. Of those who remained working, 46% showed WIS > 10 (high risk of job loss).

Conclusion: There is a large unmet need for effective therapy in AS with 65% of unselected clinic attendees meeting the ASAS criteria for biologic therapy. The criteria are robust and predict patients with worse functional status and quality of life. This makes the need for effective intervention a priority area with the aim of job retention, which is of significant economic benefit.

1088/MP

Behcet's Disease: A Case Series from Singapore

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Aim: To study the manifestations of patients with Behcet's Disease (BD) at a tertiary rheumatology center in Singapore.

Methods: The case records of patients diagnosed with BD using either the International Study Group (ISG) or the O' Duffy (OD) classification criteria, were studied. Their demographics, clinical features and outcome were recorded

Results: Thirty-seven patients were diagnosed with BD. 24 (64.9%) satisfied both ISG and OD criteria. 13 (35.1%) did not fulfill the ISG criteria but fulfilled the OD criteria. Based on the OD criteria, 7 (18.9%) were incomplete form and 6 (16.2%) the complete form. The male to female ratio was 1:1.1 (18 males, 19 females). There were 27 Chinese (73%), 7 Malay (18.9%), 2 Indian (5.4%) and 1 Caucasian (2.7%). The mean age of onset of disease manifestation was 32.7 years (range 15-58). The commonest presentations were recurrent oral ulcers (100%), genital ulcers (64.9%), joint (56.8%) and cutaneous manifestations (48.6%). Erythema nodosum (15) and papulopustular lesions (12) were the cutaneous manifestations. Systemic manifestations included arthritis (16, 43.2%), gastrointestinal manifestations (15, 40.5%) and uveitis (14, 37.8%). Recurrent abdominal pain (10, 66.7%) and diarrhoea (7, 46.7%) were the commonest manifestations of gastrointestinal BD. Gastrointestinal ulcers occurred in the ileocaecum (3), sigmoid (1) and rectum (1) of which 2 patients with ileocaecal ulcers perforated. There were only 2 cases of neuroBehcet's presenting with transient ischaemic attack/meningoencephalitis. Venous thrombosis was found only in 2 patients. Visual impairment from uveitis was the commonest cause of morbidity (25%) in our BD patients. There were no mortality.

Conclusion: BD is a rare rheumatologic condition in Singapore but its presentation and manifestations are similar to other epidemiological studies published in the literature.

1089/MP

Changing Patterns of Lupus Mortality in a Tertiary Centre in Singapore

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Aim: To compare the mortality among patients with systemic lupus erythematosus (SLE) who died in our hospital during 24-year review periods.

Methods: We retrospectively reviewed the patient and disease characteristics and causes of death of all SLE inpatients ($n = 88$) during the 24-year periods. Group A was from January 1992 to December 1995 and group B was from January 1998 to December 2001.

Results: Mortality has decreased in the recent years (54 vs 34). There were no significant differences in the gender ratio (female-to-male ratio 50:4 vs

28:6 [$p = 0.18$]), mean age at diagnosis (30.9 ± 14.1 years vs 35.6 ± 16.7 years [$p = 0.16$]) and mean age at death (36.1 ± 14.3 years vs 42.1 ± 15.4 years [$p = 0.07$]). Median disease duration was longer in group A (44 months vs 24 months). The main cause of death for both groups was infection (48.2% vs 61.8%; $p = 0.27$). In the recent years fewer patients died from active SLE alone (33.3% vs 8.8%; $p = 0.01$), but infection with active SLE accounted for proportionately more deaths (24.1% vs 47.1%; $p = 0.04$). Infection without active SLE accounted for similar proportions of death in both groups (24.1% vs 14.7%; $p = 0.4$). Atherosclerosis, malignancy and thromboembolism secondary to antiphospholipid syndrome were not the major causes of death. Other manifestations (cerebral lupus, thrombocytopenia, pulmonary haemorrhage, leukopenia, cardiomyopathy, vasculitis and serositis) were not significantly different between the 2 groups.

Conclusion: Inpatient SLE mortality has decreased in the recent years with fewer deaths from active SLE alone. However, more patients died from infection in the setting of active SLE.

1090/MP

TGF Beta Polymorphisms and Serum TGF Levels in Oriental Patients with Ankylosing Spondylitis

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Aim: To study the association between TGF β 1 gene polymorphisms and susceptibility to disease in our oriental ankylosing spondylitis (AS) patients, and to determine whether this is related to the expression of TGF β 1 in serum and in vitro peripheral blood mononuclear cell (PBMC) cultures.

Methods: Genomic DNA was extracted from PBMC of AS ($n = 70$) or unrelated healthy controls ($n = 96$). The codon 10 and 25 polymorphisms in the TGF β 1 gene were analysed using standard polymerase chain reaction (PCR)-based methods. Levels of TGF β 1 in serum were measured by enzyme-linked immunosorbent assay (ELISA). In addition, the ability of PBMC to synthesise TGF β 1 and other cytokines was assessed by in vitro cultures stimulated with mitogen.

Results: There was no statistical difference in the distribution of codon 10 and 25 TGF β 1 genotypes between AS patients and controls. However, serum TGF β 1 levels in AS patients were significantly higher than those in controls ($P < 0.001$). Incubation of AS and normal PBMC with phytohaemagglutinin (PHA) led to upregulation of TGF β 1, IL-10, TNF α and IFN γ as assessed by ELISA. Importantly, PHA-induced TGF β 1 production was significantly enhanced in AS patients when compared to normal controls whereas proinflammatory cytokines TNF α and IFN γ were reduced.

Conclusion: Our results show that AS patients expressed significantly higher levels of serum TGF β 1. Activation of AS PBMC led to enhanced TGF β 1 production accompanied by reduction of TNF α and IFN γ . These data suggest that TGF β 1 protein expression is independent of the genotype variants in our oriental AS patients.

1091/MP

A Pilot Study on the Clinical Utility of Functional MRI in the Evaluation of Suspected Neuropsychiatric Lupus

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Aim: To evaluate the clinical utility of functional MRI (fMRI) in the diagnosis of neuropsychiatric lupus (NPSLE).

Methods: Sixteen consecutive patients with SLE and suspected neuropsychiatric syndromes (classification according to the American College of Rheumatology Ad Hoc Committee on Neuropsychiatric Lupus Syndromes) underwent clinical neuropsychiatric evaluation, autoantibody testing and fMRI (DWI, FLAIR and MR-Spectroscopy).

Results: There were 3 (18.7%) males and 13 (81.3%) females, with mean age 34.2 ± 16.2 years (13-62). The mean SLEDAI (SLE disease activity) score was 18.1 ± 10.1 (2-36). Four patients were deemed not to have NPSLE after

evaluation. Of the remaining 12 patients, the manifestations were cerebrovascular disease (CVD) (stroke, transient ischaemic attack) (41.7%), psychosis (18.2%), seizure disorder (18.2%) or combinations of syndromes including the above and cognitive dysfunction or acute confusional state (27.3%). Three patients positive for anticardiolipin antibody or lupus anticoagulant all presented with CVD. None of the patients with psychosis were positive for serum anti-ribosomal P antibody. Functional MRI facilitated the diagnosis of NPSLE in patients with neurological deficits: cerebrovascular disease and seizure disorders via its ability to detect consistent neurometabolic changes and reversibility of structural changes following immunosuppressive therapies. However, fMRI was unable to facilitate the diagnosis of NPSLE in patients with psychosis or cognitive dysfunction.

Conclusion: The clinical utility of fMRI is limited to certain subsets of NPSLE manifestations. It appears to be useful in patients presenting with CVD and seizure disorders, but not with psychosis or cognitive dysfunction.

1092/MP

Primary Non-Hodgkin's Lymphoma of the Nasal Cavity: Evaluation of the Prognostic Significance of Local Tumor Extension and Combined Modality Treatment

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Aim: The aims of this study were to evaluate the impact of local tumour extension in early stage non-Hodgkin's lymphoma of the nasal cavity and to compare the treatment outcome of radiation (RT) alone with those of combined chemoradiation (CRT).

Methods: Between March 1991 and June 1997, 90 consecutive patients with Stage IE-IIIE Non-Hodgkin's Lymphoma (NHL) were diagnosed and treated in the Shanghai Cancer Hospital. Thirty-one patients have the primary disease limited in the nasal cavity (Group 1); 35 patients had disease invading 1 adjacent structure beyond nasal cavity (Group 2); and 24 patients had disease invading 2 or more adjacent structures beyond nasal cavity (Group 3). Eighty patients received combined chemoradiation and 10 patients received radiation only.

Results: The median survival for all 90 patients is 58.7 months (range 13-81 months). The actuarial 5-year overall survival (OS), disease-free survival (DFS) and local control (LC) rate were 46%, 42.5% and 92%, respectively. The extent of local disease had significant impact on OS (68.3%, 43.2% and 27.7%, $p = 0.001$), LC (100%, 90.8% and 77.5%, $p = 0.002$), and distant metastasis (11.6%, 30.4% and 51.1%, $p = 0.0001$). Multivariate analysis also showed combined chemoradiation significantly improved the 5-year OS (48.1% vs 40.2%, $p = 0.05$) and reduced the distant metastasis (27.2% vs 51.1%, $p = 0.014$).

Conclusion: Extent of local disease is a strong prognostic factor in patients with early stage nasal cavity NHL treated with combined chemoradiation or radiotherapy alone. The addition of chemotherapy to EBRT significantly improves the treatment outcome.

1093/MP

Beta-microseminoprotein in Pterygium, a Prostate Specific Protein, Highly Expressed in Ocular Surface Disease

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Aim: In microarray studies of human pterygium, an overgrowth of the cornea, we found that beta-microseminoprotein to be one of the most significantly upregulated genes when compared to normal conjunctiva tissue. We have applied PCR to validate the presence of this gene and the level of its expression for comparison to the microarray results.

Methods: One group of 8 pterygium samples and another group of control conjunctiva tissues (3 groups each with 5 pooled tissues) were prepared for

microarray analysis using the standard Affymetrix protocol. Each of the samples was hybridised into HG U133A Chip and the results were scanned 2X. The data analysis and visualisation were performed using Statistica 6.0, MAS 5.0, DMT 3.0 and GeneSpring v.5. Five additional pterygium samples and 1 conjunctiva sample (pooled normal conjunctival tissues from the same 5 patients) were prepared for PCR. The primers flanked 452bp of the 572bp total length of the gene.

Results: Data was filtered using Mann-Whitney test with p cut-off of 0.05 to determine the significance and the direction of the 'Change Call'. In 22,283 total probe sets in HG U133A Chips, 21 genes were found significantly up regulated 2 fold or more compared to the control tissue. beta-microseminoprotein increased 3.46 times (SLR = 1.79) on average compared to normal conjunctiva. PCR confirmed the up regulation of the gene in pterygium compared to normal conjunctiva.

Conclusion: This novel gene may be uniquely associated with the mucosal tissue of the ocular surface and signal the development or growth of pterygium.

1094/MP

Muscarinic Acetylcholine Receptors (mAChR) Regulate proliferation of Human Scleral Fibroblasts (SFs)

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Aim: Atropine sulfate, a muscarinic antagonist has been shown to retard axial elongation in experimentally induced myopia and in clinical myopia. Muscarinic receptors appear to affect human and mouse scleral fibroblasts (SF); however, their role in regulating cell physiology is not clear. We have investigated the effect of muscarinic agents on human SF.

Methods: Human SF, used between passages 2-4. SFs were plated into 96 well plates containing DMEM + 10% FBS, after being allowed to attach, they were serum starved overnight. SFs were treated with either a muscarinic antagonist (atropine, pirenzepine) or agonist (carbachol) at 0.1, 1, 10, 100 μ M. Bromodeoxyuridine (BrdU) was added to the fibroblast culture at the same time as the test agent, to be incorporated into newly synthesised DNA. DNA synthesis was measured after 24-hour incubation. BrdU incorporation into SF was measured by ELISA.

Results: BrdU incorporation into SF was inhibited by the presence of atropine or pirenzepine, a general muscarinic antagonist (0.1, 1, 10, 100 μ M) in a dose dependent manner ($p < 0.05$, ANOVA, $n = 4$). In contrast, BrdU incorporation of SF was found to increase in a dose dependent manner with carbachol ($p < 0.05$, ANOVA, $n = 4$). Atropine was able to block the effect of carbachol when SFs were pre-treated (3 hours) with an equimolar amount of atropine ($p < 0.05$, ANOVA, $n = 4$).

Conclusion: Our results suggest that the muscarinic receptors exist on human SF and are involved in the regulation of cell proliferation. Their effects may be mediated through G-protein linked receptor in the SF membrane.

1095/MP

Effect of Muscarinic Antagonist (Atropine) on Experimental Model of Myopia in the Mouse

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Aim: The purpose of this study was to use form deprivation to develop experimental myopia in the mouse and to use this model to investigate the effect of atropine in vivo.

Methods: A negative (-10D) contact lens was attached over the right eyes of 80 mice. Two experiments were carried out with 20 mice in the atropine treatment group, and 20 mice in the control group for a total of $n = 40$, and $n = 40$ for saline control group. The atropine group received a single sub-conjunctival injection (12 μ l) of atropine (1 μ M) a day for 14 days, and the control eye received 12 μ l of normal saline. After 14 days eyes were refracted, axial length measured with a digital calliper, the eye embedded in plastic.

Results: Axial length of myopic eyes receiving saline was 3.811 ± 0.005 mm and the refractive error -4.75 ± 0.004 , while myopic eyes receiving atropine were 3.577 ± 0.007 mm long, and the refractive error was 1.5 ± 0.006 , the control eyes for these groups were 3.439 ± 0.005 mm, $6.25D \pm 0.007$, and 3.467 ± 0.007 mm, $6D \pm 0.007$ respectively ($p < 0.05$, ANOVA, $n = 80$).

Sclera thickness increased from anterior to posterior in atropine myopic eyes and control eyes. In NS group myopic eyes, sclera was thinner at equator and posterior.

Conclusion: Present study shows that atropine reduces the experimentally induced myopia in the mouse. The mouse may be a promising model for the analysis of myopia. We are continuing the molecular analysis of this potentially useful new model.

1096/MP

In vitro Studies of Lactobacillus on Bladder Cancer Cells

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Aim: Previous studies with Lactobacillus rhamnosus strain GG (LGG) demonstrated that LGG is capable of eliciting anti-tumour activities through a proteinaceous product. This series of studies serve to further evaluate and characterise the cytotoxic protein(s).

Methods: 1×10^5 cells/ml murine bladder cancer cells, MB49, were incubated with 1×10^8 and 1×10^9 colony forming units/ml LGG for 72 hours before the spent culture supernatant is harvested. The harvested supernatant was filtered first before Proteinase K (Sigma) treatment (1 unit/2 ml). In a separate experiment, the filtered spent supernatant was subjected to size fractionation using centrifugal filter devices (10 kD, 30 kD, 50 kD) (Millipore). Following Proteinase K and filter fractionation, 100 ml of the supernatants were separately inoculated into freshly cultured cells (1×10^4 cells/100 ml) in 96 well plates. After 72 hours, the number of living cells in culture was determined with the MTS colorimetric method (Promega). Two-dimensional gel electrophoresis was also used to identify and determine the protein profile in the culture systems following LGG treatment. The results obtained were statistically analysed with one-way ANOVA.

Results: Cells incubated with Proteinase K-treated supernatant proliferated as well as control cells. However, the wells incubated with untreated spent supernatant had significantly fewer cells than the controls. The cytotoxic effect was also found to be dose-dependent. In the filter fractionation experiment, most activity was observed in the less-than-10 kD fraction, where these wells had significantly fewer cells than the controls.

Conclusion: The results obtained confirm the presence of a cytotoxic protein and strongly suggest that it is less-than-10 kD in size.

1097/MP

Prospective Study on Delays in the Diagnosis and Treatment of Tuberculosis

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Aim: We determine the extent of diagnostic delay for infectious tuberculosis (TB) patients referred to TB control unit (TBCU) and to identify patient factors and points of delay.

Methods: 87 symptomatic patients started on TB treatment at TBCU from April to October 2002 were interviewed. Data were collected on nature and duration of symptoms before first medical consultation, number of medical consultations and doctors seen, duration of symptoms before chest x-ray was done.

Results: The median patient delay (time from onset of symptoms to medical consultation) was 2 weeks (range 1-52 weeks), 4 weeks for smear positive compared to 1 week for smear negative patients. The median patient delay was significantly longest at 6 weeks for those aged 20 to 29 years (Mann Whitney U Test, $p 0.049$). Patient delay did not vary by gender, ethnic group, nature of symptoms and nationality. At 4 weeks of symptoms, 62% had a chest X-ray done. The median health care system delay (time taken from first medical consultation to start of treatment) was 21 days. There was no significant delay on health care system by AFB smear status and chest X-ray

findings. At TBCU, 59% of patients were started on treatment on first consultation and by 2 weeks, 95% were on treatment. The median time from onset of symptoms to treatment was 51 days.

Conclusion: There is a delay in the diagnosis of tuberculosis, particularly at primary health care level. Primary care physicians should be made more aware of the disease so that treatment would not be delayed.

I098/MP

Development of Free Statistical Software Enabling Researchers to Calculate Confidence Levels, Clinical Significance Curves and Risk-benefit Contours

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Aim: Confidence levels, clinical significance curves, and risk-benefit contours are tools improving analysis of clinical studies and minimizing misinterpretation of published results, however no software has been available for their calculation. The objective was to develop software to help clinicians utilize these tools.

Methods: Excel 2000 spreadsheets were designed for use in studies comparing 2 patient groups. The workbook was protected and encrypted so that users can modify only input cells.

Results: The workbook has 4 spreadsheets. Sheet-1 comprises instructions and graphic examples for use. Sheet-2 allows the user to input study results (e.g. survival rates) into a 2-by-2 table. Confidence intervals (95%), p value and the confidence levels are automatically generated. An additional input cell allows the user to determine the confidence associated with a specified level of benefit; thus if the user wishes to know the confidence that Treatment A is truly at least 10% better than B, 10% is entered. Sheet-2 automatically displays clinical significance curves, graphically illustrating confidence levels for all possible benefits of one treatment over the other. Sheet-3 allows input of toxicity, determining the confidence that the relative toxicity of the most effective arm does not exceed user-defined tolerability. Sheet-4 automatically calculates risk-benefit contours, displaying the confidence associated with a specified scenario of minimum benefit and maximum risk of 1 treatment arm over the other. The spreadsheet is freely downloadable at http://www.theshakespeares.com/confidence_calculator.html

Conclusion: A simple, self-explanatory, freely available spreadsheet calculator was developed. The incorporated decision-making tools can be used for data-analysis and improve the reporting of scientific research results.

I099/MP

Radiation Oncology Professional Revalidation: Is it the Instrument and Not the Doctor that First Needs to be Valid?

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Aim: The Royal Australian and New Zealand College of Radiologists (RANZCR) developed a draft audit checklist for testing as a competency assessment instrument for Radiation Oncologists (ROs) in Australia, New Zealand and Singapore. We evaluated the reliability of this instrument as well as our own The Cancer Institute (TCI) designed instrument.

Methods: Competency instruments: The RANZCR instrument contains 11 competency items scored using various scoring categories. The TCI-Singapore instrument includes 19 competency items scored as "adequate" or "inadequate". Evaluation: Two ROs performed independent chart review of 80 randomly selected patients seen in TCI Radiation Oncology. Both RANZCR and TCI-Singapore instruments were used to score each chart. One week later 1 RO repeated the process. Instruments were compared for inter- and intra-observer reliability using misclassification rates

Results: Misclassification of RANZCR items ranged from 0-39% (median 8%) for intra-observer reliability, and 0-81% (median 9.5%) for inter-observer reliability. For example, inter-observer reliability for non-radiation management items ranged from 69 to 81% misclassified, and "Examination" and "Dose ICRU" items were 46% and 38% respectively. The TCI-Singapore instrument demonstrated higher reliability, with misclassification rates ranging

from 0 to 7.5% for both intra- and inter-observer reliability (median 2.5% and 1.3%, respectively).

Conclusion: Ensuring revalidation instrument validity is critical to reliably judge clinician competency. The draft RANZCR instrument is not valid in its present form and requires modification. The TCI-Singapore instrument demonstrated higher reliability, maintains relevance for competency assessment, and has the added advantage of incorporating a simple scoring system and criticality index to allow comparisons against future college standards.

I100/MP

Evaluation of the Efficacy, Participant Satisfaction and Cost-effectiveness of an Oncology Continuing Medical Education (CME) Programme

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Aim: Audit with feedback (AWF) is considered effective CME, however educational efficacy for oncologists is unknown. We evaluated efficacy, cost-effectiveness and participant satisfaction of an AWF-CME intervention for radiation oncologists (ROs).

Methods: CME programme: incorporated fortnightly random patient chart audit, scoring management adequacy via checklist. Scores and educational feedback were provided at a same-day institutional meeting. Evaluation: RO behavior/performance was evaluated via chart review of new patients seen 2 months before commencement of AWF-CME (T0), and after 12 months (T1). A validated instrument scored 19 items as "0" (inadequate/inappropriate) or "1" (adequate/appropriate). We compared mean point-score for 10 behaviour items targeted by the AWF-CME checklist, 3 non-targeted behaviour items, and 6 performance items; also percent charts scoring "1" for each item at T0 vs. T1. A 14-item questionnaire measured participant satisfaction before and after efficacy results were known. Annual cost and cost-per-point gained incorporated salaries of organizers/participants, and consumables.

Results: 113 and 118 charts were evaluated at T0 and T1. Targeted behavior improved between T0 and T1 (8.7 to 9.2 out of 10, $p = 0.0001$), with no change for non-targeted behavior/performance items. Improvement occurred for targeted items "Letter to referrer" (percent charts scoring "1" increased from 53% to 66%, $p = 0.04$), "Treatment intent" (54% to 77%, $p = 0.0002$), "Laterality doubler" (91% to 98%, $p = 0.04$), and "Isodose-plan signed" (94% to 100%, $p = 0.006$). Improvements varied between ROs. Participant satisfaction was positive, increasing after efficacy result distribution ($p = 0.0001$). Annual costs and cost-per-point gained were \$13,820 and \$27.

Conclusion: Audit with comparative, educational feedback is cost-effective and positively-perceived CME, significantly improving targeted RO behaviour.

I101/MP

Outcome of Treatment of Multi-drug Resistant Tuberculosis at the Tuberculosis Control Unit, 1988-2000

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Aim: We evaluated the outcome of multi-drug resistant tuberculosis (i.e., resistance to at least rifampicin and isoniazid) (MDR TB) cases treated at the Tuberculosis Control Unit (TBCU) from 1988 to 2000.

Methods: Data on MDRTB patient demographics, disease characteristics, treatment course and outcome were obtained from the TBCU case records.

Results: 61 out of 65 case records were available for analysis. There were 42 males, 19 females; age range 17 to 90 years (median 48.5 years). 60.7% were Chinese, 9.8% Malays, 6.6% Indians, 1.6% other races. 21.3 % were foreigners. 61% were new cases, 39% were relapse cases. All but 1 patient had pulmonary TB. 28% had diabetes mellitus, 5% were HIV-infected. Foreigners had significantly more far advanced disease with cavity as compared with residents (61.5% vs 14.6%, OR 9.3, 95% CI 1.9-47.6, $p < 0.001$). The patients were resistant to a median of 3 drugs. Median time to sputum culture conversion was 1.73 months. Treatment was continued for a median of 17 months after culture conversion. The most common adverse effect was gastro-

intestinal side effects (33%). 32 (52%) patients completed treatment, 6 died of TB, 9 died from other causes, 7 left the country, 7 defaulted treatment. One patient relapsed 2 years later and was re-treated successfully. Factors associated with successful treatment completion were age <60 years (OR 4.7, 95% CI 1.22-18.9, $p < 0.002$), weight > 55 kg (OR 3.5, 95% CI 1.08-14, $p < 0.03$), and resistance to <3 drugs (OR 4, 95% CI 1.18-14.2, $p < 0.02$).

Conclusion: Patients with MDR TB can be cured with appropriate treatment.

1102/MP

The M3 Muscarinic Receptor is Expressed in Human Limbal and Conjunctival Epithelial Cells

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Aim: Muscarinic cholinergic receptors are found on non-neural cells and they can be involved in regulating many cell functions. This study was to determine whether the acetylcholine muscarinic receptor subtype 3 (MR3) exists in human limbal and conjunctival epithelial cells.

Methods: Human limbal and conjunctival epithelial cells were isolated and grown in culture medium (SHEM for limbal epithelial cells in the presence of mitomycin C-treated 3T3 feeder cells, KGM for conjunctival cells). MR3 was detected using FITC immuno-fluorescence on the cultured cells (anti-MR3 from Research and Diagnostic Antibodies). Upon confluence, cells were homogenised and subjected to SDS-PAGE electrophoresis and western blot to detect MR3 protein expression.

Results: Staining for the MR3 receptor was detected on the membrane and in the cytoplasm of limbal and conjunctival epithelial cells. Western blot analysis revealed the presence of immunoreactive bands for MR3 muscarinic receptor in early passage limbal and conjunctival epithelial cells.

Conclusion: Muscarinic receptor subtype MR3 is present on cultured human limbal and conjunctival epithelial cells. This suggests the release of acetylcholine from parasympathetic nerves may play an important role in regulating the growth and differentiation of human ocular surface cells.

1103/MP

Proteomic Analysis of Human Tears: In Response to Ocular Surface Injury

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Aim: The objective of this study is to determine the qualitative and quantitative changes of tear protein in response to ocular surface injury.

Methods: Human tears were collected from the patients prior to ocular surface surgery and on day 1, 3 and 30 after surgery and were rapidly profiled using SELDI-TOF ProteinChip. Candidate proteins were subjected to tryptic digestion, and the resulting proteolytic peptide fragments were analysed by Q-TOF mass spectrometer. LC-ESI-MS was used to quantify the levels of human Alpha-defensins (HNP-1 and HNP-2) in human tears.

Results: With this approach, one of the obvious changes we found was that a group of human Alpha-defensins (HNP-1, HNP-2 and HNP-3) was significantly up-regulated after ocular surface surgery. Further confirmation of the identity of the Alpha-defensins in human tear fluids was made by LC purification, trypsin digestion and ESI-MS/MS analysis of their tryptic digests. The average concentrations of HNP-1 and HNP-2 in normal control tears were 0.36 ± 0.26 ug/ml ($n = 13$) and 0.28 ± 0.28 ug/ml ($n = 16$) respectively. After surgery the levels of HNP-1 and HNP-2 significantly increased to 6.52 ± 2.40 ug/ml ($P < 0.0000$) and 4.49 ± 1.68 ug/ml ($P < 0.0000$) at postoperative day 1 and 15.59 ± 6.28 ug/ml ($P < 0.0000$) and 7.16 ± 4.56 ug/ml ($P < 0.0000$) at postoperative day 3.

Conclusion: The marked increase in the level of HNP-1, HNP-2 and HNP-3 after ocular surface surgery suggests human alpha-defensins play an important role protecting the ocular surface from microbial attack and could modulate ocular surface wound healing processes.

1104/MP

UVB Irradiation Induces the Oxidative Stress Response and Cytokine Production in Human Conjunctival Cells

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Aim: To examine the response of cultured human conjunctival cells to UVB irradiation, the production of cytokine, the activation of cell signalling pathways and oxidative stress response.

Methods: Primary cultured human conjunctival cells at passage 2 to 3 were serum starved for 5 hrs and subsequently exposed to UVB at a dose of 20 mJ/cm². Culture medium and cells were harvested at different time intervals to assay for the activation of Nrf2, MAP kinases and production of IL-1a.

Results: Nrf2 protein level increased 10 minutes after UVB exposure. p38 MAP kinase was maximally stimulated 30 mins after UVB exposure, and attenuated after 1 hr. A similar time course of activation was also observed for c-Jun Kinase (JNK). p42/44 MAP kinase and Akt upregulation occurred at around 10 mins after the UVB irradiation. IL-1a in the medium increased from 0.3pg/ml in control cells to 20 pg/ml at 24 hrs after the UVB exposure.

Conclusion: The accumulation of Nrf2 suggested that the effect of UVB on human conjunctival cells may be mediated through oxidative stress. UVB exposure of human conjunctival cells leads to the activation of various MAP kinases including p38, p42/44 kinases as well as c-jun kinase. It also induced the accumulation of Nrf2. The activation of such kinases may be responsible for the production of inflammatory cytokines.

1105/NUR

Perceptions of Epilepsy Patients Towards Their Quality of Life

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Aim: To assess the quality of life of patients with epilepsy in Singapore.

Methods: 335 epilepsy patients seen at Outpatient Epilepsy Clinic between November 2001 and January 2003 were interviewed by an epilepsy nurse clinician using a structured questionnaire adapted from questionnaires used by regional epilepsy societies.

Results: 160 patients (47.8%) were male and 175 (52.2%) were female. The average seizure duration was 16.5 years (range 0.5 to 55 years). 192 (57.3%) patients have at least one but less than 12 seizures per year, 94 (28.1%) have at least 1 seizure per month and 42 (12.5%) have at least 1 seizure per week. 66.1% patients have generalised or secondary generalised seizures while 33.9% patients have only partial seizures. 21 (6.3%) patients were undergoing full time education, 119 (35.6%) were unemployed, 7 (2.1%) were attending sheltered workshop or training centre, 33 (9.9%) were working part-time and 155 (46.3%) were working full-time. 136 (42%) patients have no income at all and only 75 (23.1%) patients have income between S\$1001 and S\$2000. 155 patients (46.3%) cited work-related problems as the major factor impacted by epilepsy. Family problems (28.4%), self-esteem (26.3%) and being unable to live independently (25.7%) were also cited. Most patients expressed satisfaction with their drug treatment with most quoting little side effects significant enough to affect work performance or daily life.

Conclusion: People with epilepsy have significant quality of life impairment. Efforts to improve employability and relationship skills may help to enhance quality of life for people with epilepsy.

1106/NUR

A Retrospective Study Examining the Prevalence Wound Infection Rates and Explore its Determinants in the Polyclinic Setup

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Aim: To examine the prevalence of community acquired wound infection at NHG Polyclinics and explore its determinants.

Methods: A retrospective study comprising 1000 consecutive patients from Toa Payoh and Hougang Polyclinics attending for wound dressing procedures between January and September 2002. Patients with hospital-acquired infections and high-dose oral steroids were excluded. Using the standardised

NHGP wound chart, data such as demographics, solutions, co-morbidities were collected. Wounds were categorised as infected guided by CDC criteria. Results were analysed using SPSS.

Results: Infection prevalence rate was 1.7% (95% CI 1.1% - 2.8%), with no significant differences between gender ($p = 0.436$), ethnic groups ($p = 0.964$), diabetic status ($p = 0.146$) and the types of solutions used ($p = 0.318$). Interestingly, 15% of patients were prescribed chlorhexidine as the wound-cleansing agent. For cases with infection, the median of infection free days was 7, mean (SD) 8.1 (5.3), range 3 to 24 days. Older patients were more likely to have wound infection ($p = 0.031$) and diabetics with infected wound are more likely to be readmitted to hospital ($p < 0.001$, RR 71.4, 95% CI 41.7 to 125.0).

Conclusion: Our wound infection prevalence rate was 1.7%. There is a need to educate staff about the appropriate use of dressing solutions. Although no significant difference in infection rates was demonstrated with different solutions, including normal saline, which is the recommended cleaning agent, further studies are required. We propose to set up an infection registry aim at detecting patients at risk of wound infection to improve quality care.

I107/NUR

A Survey of COPD Patients' Knowledge of Their Disease and Treatment in a General Hospital in Singapore

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Aim: To investigate the level of patients' knowledge of the cause of their illness among hospitalised patients with chronic obstructive pulmonary disease (COPD) prior to the implementation of a hospital-wide clinical management pathway including nurse-provided education.

Methods: Consecutive patients admitted to general medical wards for acute exacerbation of COPD were interviewed by a nurse clinician on their knowledge of the cause of the illness, use of medication and oxygen therapy.

Results: 103 patients (91 male, mean age 72, mean FEV1 39.8 + 13.2 percent predicted) were included. Six patients had severe, 41 moderate and 56 mild exacerbations according to the Anthonisen severity scale. The median duration of disease from diagnosis was 3 years (range 1-20). 22 patients were active smokers while the rest were ex-smokers. 63 patients (61.2%) did not consider cigarette smoking to be a cause of their illness and among them, 14 were still smoking. All patients were using metered dose inhalers but only 37.5% of them could demonstrate faultless inhaler techniques. Four patients were using volumetric spacers and 3 of them could demonstrate proper use of spacer technique. Seventeen patients had home nebulizers but only 3 could demonstrate proper use of nebulizers. Eleven patients were on long-term oxygen therapy but only 6 reported using oxygen > 15 hours/day.

Conclusion: In this survey of patients with recurrent hospitalisation for COPD, most patients did not attribute their illness to cigarette smoking. Patients' knowledge and use of inhalers, home nebulizers and oxygen therapy were sub-optimal in most cases. These findings form the basis for provision of routine intensive patient education among patients hospitalised for COPD.

I108/NUR

A Comparison of Patient and Nurse Ratings of Pain Level in Postoperative Orthopaedic Patients.

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Aim: 1. To compare patient's self-rating of pain level with that of the registered nurse's assessment in postoperative orthopaedic patients. 2. To describe registered nurses' knowledge, perceptions and attitudes towards pain management in postoperative patients.

Methods: This is a descriptive study. It was conducted in 7 orthopaedic units in Tan Tock Seng Hospital. 141 adult patients listed for elective orthopaedic surgery from 1 October 2002 to 31 December 2002 were recruited for part one of the study. Using Visual Analogue Scale (VAS) pain rating scale and Wong-Baker Facial Coding System (FCS) patient's self-rating of pain and facial expression was compared with that of the registered nurse. In part two, 102 registered nurses working in the orthopaedic units participated in the study. A 50-item questionnaire survey comprising a 5-point scale was

designed to describe the registered nurses' approach to acute pain, pain management and administration of analgesia of postoperative orthopaedic patients.

Results: Wilcoxon-Signed Ranks Tests and reliability analysis was used in part one and descriptive statistics in part two of the study. The results revealed significant differences between the way patients and nurses measured their pain levels ($r = 0.0764$).

Conclusion: We hope this study could be used to reinforce the significant role played by nurses in the optimal management of postoperative patient's pain.

I109/POD

Incidence of Positive Direct Antiglobulin Test (DAT) and its Clinical Significance at an Acute Care Hospital, Singapore

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Aim: A retrospective study was done on all patients who gave a positive result for the direct antiglobulin test (DAT), its incidence and severity of haemolysis.

Methods: Pre-transfusion records of patients admitted between the period Year 2001 and 2002 were reviewed. The transfusion history and test results of the patients were then retrieved from the laboratory information system. The results, in particular, the haemoglobin, the concentration of bilirubin and lactate dehydrogenase (LDH) were collated and tabulated.

Results: 137 patients had a positive result for the DAT. 39% had their red blood cells coated with IgG and complement, 39% coated with IgG alone and the remaining 22% coated with complement alone. 47% had a haemoglobin level of less than or equal to 8.0 g/dL. 43% (26/60) of the patients tested for bilirubin had elevated levels. 63% (33/52) of the patients tested for LDH had elevated levels of more than 600 U/L. 82% gave a positive result for the pre-transfusion antibody screen. 9% had no clinically significant antibodies detected while another 9% had no pre-transfusion antibody screen performed, as transfusion had not been indicated for them. 55% of those who gave a positive result for the antibody screen were identified as having a non-specific autoantibody, 24% as having an autoantibody (with e and I specificity), 11% as having an alloantibody together with an autoantibody and the remaining 10% with just an alloantibody.

Conclusion: Positive DAT is not uncommon in clinical practice. Majority of the patients do not show signs of haemolysis and usually identified as having non-specific autoantibodies.

I110/POD

Pulmonary Rehabilitation for Chronic Lung Disease Other Than COPD

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Aim: The role of pulmonary rehabilitation (PR) in improving the functional status of patients with chronic obstructive pulmonary disease (COPD) is well established. However, the benefits of PR in patients with other chronic lung disease (CLD) have not been proven. We reviewed the outcomes of a pulmonary rehabilitation programme for patients with CLD other than COPD.

Methods: All patients underwent lung function and exercise testing before and after undergoing a 6-week, outpatient, hospital-based PR programme. A major component of the programme was supervised exercise training 3 sessions per week, lasting 60 minutes each, with the training intensity maintained at near-maximal targets.

Results: Seventeen patients (12 male, mean age 59) with the following conditions were included: bronchiectasis (9), obesity-hypoventilation syndrome (2), post-pleurodesis for recurrent pneumothorax (2), interstitial lung disease (1), rigid spine syndrome (1), fibrothorax (1), bronchiolitis obliterans (1). There was no significant change in lung function in all patients after pulmonary rehabilitation. Maximal oxygen uptake and work-rate improved significantly after PR by 242 mL.min⁻¹ ($p = 0.03$) and 17 W ($p = 0.006$) respectively. Six minute walk tests improved significantly by a mean

of 37.1 m ($p = 0.024$). Borg dyspnoea scores at maximum exercise decreased from 4.2 to 3.8.

Conclusion: Patients with CLD other than COPD can improve their exercise capacity by undergoing PR. Further studies are required to define selection criteria and optimise exercise prescription in this category of patients.

I111/PD

D-dimer: A Simple and Useful Test for Exclusion of Clinically Suspected Deep Vein Thrombosis

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Aim: Deep vein thrombosis (DVT) is a common disorder associated with significant morbidity and mortality. Symptoms are non-specific and radiological procedure based on compression doppler ultrasonography (CUS) is commonly used for the diagnosis of DVT. D-dimer, a cross-linked fibrin degradation product is elevated in DVT but also in other clinical situations common in hospitalised patients. D-dimer is more useful for exclusion of DVT due to the high sensitivity and negative predictive value of the test. We evaluated the use of Liatest D-dimer for exclusion of DVT.

Methods: Liatest D-dimer was performed on 77 plasma samples of in- and outpatients with clinically suspected DVT. CUS was used as the reference method to confirm the diagnosis of DVT. Accuracy indices (sensitivity, specificity, negative and positive predictive values) calculated using the Receiver Operating Curve were analysed.

Results: DVT was diagnosed in 25 of 77 patients by CUS. 55 of them have D-dimer level above the cut-off of 0.5 ug/mL (prevalence 71.4%). The sensitivity and negative predictive value of the test was high at 92.0% and 90.9% respectively whilst specificity and positive predictive value were low at 38.5% and 41.8%. There were 2 false negative results.

Conclusion: With low specificity but high sensitivity, Liatest D-dimer assay is a useful test for exclusion of clinically suspected DVT. It is probably best used for screening in the outpatient setting where DVT prevalence and risk for DVT of presenting patients are lower, improving its negative predictive value. This may reduce hospital admission and unnecessary radiological investigations.

I112/PD

Smart Doctors Collect Urine for Culture Before Starting Antibiotics

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Aim: To quantify the impact of administering antibiotics before urine collection on the value of urine culture.

Methods: 545 urine specimens submitted for routine urine culture from inpatients on the day of admission or 1 day after the day of admission were tested for the presence of antibiotics. All urine specimens received on Tuesdays, Wednesdays and Thursdays over a period of eight weeks were tested. 10 µl of urine was streaked onto Mueller Hinton agar plates that were lawned with *S.aureus* ATCC 25923 and *E.coli* ATCC 25922. A zone of inhibition with either organism was considered positive for the presence of antibiotics. The presence of antibiotics was correlated with the culture result.

Results: We detected antibiotics in 51% of urines tested. Of the urines that tested negative for antibiotics 23% had $\geq 10^5$ cfu/ml. Of the urines that tested positive for antibiotics only 5% had $\geq 10^5$ cfu/ml implying a loss of 78% of cultures with $\geq 10^5$ cfu/ml. Among urines positive for antibiotics 79% (220/279) had no significant bacterial growth. Among urines negative for antibiotics 50% (132/266) had no significant bacterial growth implying an increase of false negative cultures by about 58%.

Conclusion: Administering antibiotics before urine collection leads to the loss of 78% of cultures with $\geq 10^5$ cfu/ml. This loss leads to clinical confusion, unnecessary investigation, treatment and prolonged hospital stay. Urine samples for culture should be collected in the admitting clinics/emergency department before starting antibiotics and should not be postponed for later collection in the wards.

I113/PD

The Effect of Psychosis Patients and Families' Causal Beliefs on Duration of Untreated Psychosis

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Aim: Studies have examined the reasons why people with psychosis delay seeking psychiatric treatment. In this retrospective study on the Singaporean Chinese population, impact of causal beliefs about psychosis on the duration of untreated psychosis (DUP) is examined. It was expected that a dominant belief system, or a combination of these beliefs would best predict DUP. Indigenous Chinese beliefs were also expected to be the strongest predictors of long DUP. Socio-demographic factors were also considered.

Methods: Participants comprised patients and their families from the Early Psychosis Intervention Programme. Causal beliefs were assessed through the Opinions about Psychological Problems – Modified (OPP-M) questionnaire which constitutes beliefs derived from 3 causal models: indigenous Chinese model (dang-ki, feng-shui and Chinese medicine beliefs), Western psychological model (psychodynamic, behavioural, humanistic and cognitive beliefs), and the 'other' model (organic, socio-economic and naïve beliefs).

Results: Overall, findings were mixed. A linear combination of patients' cognitive and dang-ki beliefs and years of education jointly predicted DUP. Stronger endorsement of cognitive belief and less education were significantly associated with shorter DUP. Unexpectedly, stronger endorsement of indigenous Chinese beliefs were insignificantly associated with shorter DUP. Family beliefs in feng-shui and possibly, organic causes, predicted DUP. Stronger endorsement of these beliefs were associated with shorter DUP.

Conclusion: The unexpected finding of a negative trend between patients' endorsement of Chinese beliefs and DUP suggests the involvement of mediating factors in the relationship between causal beliefs and DUP. Future research should examine these factors and the impact of causal beliefs on outcome measures such as treatment compliance.

I114/PD

Neurocognition and Duration of Untreated Illness

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Aim: Studies have suggested that untreated psychosis can have a toxic effect on the brain. This study investigates the relationship between duration of untreated illness (DUI) and neurocognitive performance in patients with first-episode psychosis.

Methods: Patients included are aged between 15 to 40 with first-episode psychosis. DUI is defined as the period between onset of any psychiatric symptoms to initiation of treatment and assessed from structured interviews with patients and relatives. All patients were administered a neurocognitive battery. The National Adult Reading Test (NART) estimates pre-morbid cognitive functioning whereas the Raven's Progressive Matrices (RPM) estimates current cognitive functioning. The Continuous Performance Test (CPT) and Digit Span subtest from the Wechsler Adult Intelligence Scale (WAIS – III) are measures of attention while the Trail-making Test and the Wisconsin Card Sort Task (WCST) are measures of executive function.

Results: Forty one patients with mean age of 25.1 (± 5.7) years and mean DUI of 20.5 (± 27.0) months, median of 11.0 months, and range of 0 to 120 months, participated in the study. Fifteen patients (36.6%) were female. Spearman's partial correlation coefficients were used to examine the relationships between DUI and tests in the neurocognitive battery, controlling for years of education, age and gender. These did not achieve statistical significance.

Conclusion: The absence of significant correlations between DUI and neurocognitive functioning suggests that psychosis may not exert a toxic effect on brain functioning. However, further research using sophisticated brain imaging may allow greater exploration.

1115/PD

A Comparison Study between Vitek 1 and Vitek 2 Automated Systems in a Clinical Set-up

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Aim: The Vitek 1 automated system has been used in the National University Hospital for the past 3 years. The system has shortened reporting time, simplified and standardised workflow, and provided test information which, when used in conjunction with other systems, assist in completion of test results. Vitek 2 is a new version with increased automation. It comes with an Advanced Expert System for analyzing organism's profile and makes use of fluorescent technology for bacterial identification. The purpose of this study was 3-fold: to identify the problems that may be encountered during routine work, to determine additional tests required for complete identification of organisms and to work out the most appropriate workflow for our laboratory.

Methods: The trial study was separated into 2 parts. Part 1: A selection of organisms was used to challenge the system. Susceptibility testing was done on thirty-four multi-resistant strains and identification testing on over one hundred bacterial and yeast strains. Discrepancies encountered during identification and susceptibility testing were noted as major and minor errors according to the seriousness of the problem. Part II: One hundred isolates from routine cultures were tested in parallel by the Vitek 1 and Vitek 2 systems.

Results: Significant discrepancies were noted in the susceptibility results of amikacin when tested against *A. baumannii*. The 2 systems showed agreement when testing common clinical isolates.

Conclusion: Overall, the Vitek 2 system has proven to be not only rapid but is able to analyse intelligently, assisting laboratory workers in making more informed decisions.

1116/PD

The Effects of a Low Glycaemic Index cum High Fibre Diet on the Metabolic Control of Patients with Type 2 Diabetes Mellitus

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Aim: Medical nutrition therapy is essential in successful diabetes management. The aim is to investigate whether a low glycaemic index (GI) cum a high fibre diet could result in better glycaemic, lipid, and weight control in patients with Type 2 diabetes mellitus (DM).

Methods: Overweight (BMI >23 kg/m²) subjects (n = 26) with suboptimal glycaemic control (HbA1c 8-10%) recruited from the Alexandra Hospital Diabetes Centre were randomised using a 2² factorial design into 4 diet groups — (Grp 1) high GI + low fibre, (Grp 2) high GI + high fibre, (Grp 3) low GI + low fibre, (Grp 4) low GI + high fibre. They were given the respective dietary advice, asked to keep a 3-day food record and to monitor their blood glucose thrice weekly throughout the 3-month study period. Subjects are then followed up at 4th, 10th and 16th week to reinforce diet and to review various parameters.

Results: No significant differences in the baseline characteristics were found among groups. Using multiple comparisons, Grp 4 was observed to have a significant reduction in BMI (D4 = 3.9% vs. D1 = -0.4%; p = 0.015) and improvement in HbA1c (D4 = 16.9% vs. D2 = -4.5%, p = 0.017) when compared to Groups 1 and 2 respectively. Compliance with dietary food record appeared to correlate with improvement in HbA1c.

Conclusion: Low GI cum high fibre diet appears to help Type 2 DM subjects with better glycaemic and weight control, but not with lipids. Dietary food record may be useful for diabetes management.

1117/PD

Excess Amniotic Fluid Supernatant: A Novel Source of DNA for Prenatal Genetic Testing

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Aim: Amniotic fluid (AF) is the most common tissue used in prenatal diagnosis. AF supernatant is normally discarded after separation of cellular material for culture by low speed centrifugation. We sought to develop and validate a method of extracting DNA from leftover supernatant AF to extend its diagnostic potential and avoid wastage of fetal tissue.

Methods: 300 AF samples (13-23 weeks gestation) were studied. DNA was extracted from cellular material obtained after high speed centrifugation of AF supernatant and subjected to multiplex-PCR using 3 polymorphic chromosome 21 markers. Fragment separation and analysis of PCR products was carried out on a MegaBACE DNA Analyser (Amersham Biosciences, NJ, USA). Peak height fluorescent intensity ratios were used to predict allele copy numbers in normal and Down Syndrome (DS) samples.

Results: Detectable DNA at concentrations ranging from 27-895 ng/μl was obtained from all AF samples tested. A trend towards higher DNA yield (p <0.001, Spearman correlation coefficient = 0.69), but lower purity (p <0.001, Spearman correlation coefficient = -0.48) was observed with increasing gestational age. Only 2.33% (7/300) of samples showed amplification failure in 1 or more markers; failure was associated with a low (<50 ng/μl) DNA concentration (p <0.001, Fisher's exact test). PCR was invariably successful at a DNA concentration of ≥50 ng/μl. The peak height ratio of resultant PCR products allowed subsequent detection of DS in all abnormal cases.

Conclusion: AF supernatant is a viable, novel, alternative source of DNA which may be used for prenatal detection of trisomic disorders such as DS. This method may also be extended to molecular investigation of other genetic disorders.

1118/PD

Comparative Evaluation of Weil Felix Test with the Indx Custom Dip-S-Ticks (DS) for the Detection of Arthropod-borne Diseases

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Aim: Rickettsial diseases are widely distributed throughout the world and continue to be an important health problem. They are probably underdiagnosed. The Weil Felix test which is widely used has been shown to have poor sensitivity. The DS is a new rapid test using an enzyme immunoassay (EIA) dot technique for the detection of antibodies to *R. rickettsii*, *R. typhi*, *O. tsutsugamushi* (Karp strain), and *O. tsutsugamushi* (Kato and Gilliam strains). In this study, we compared the DS with the Weil Felix.

Methods: 280 serum samples were selected from patients tested with the Weil Felix. The sera were re-tested using the DS. The sensitivity and specificity of the Weil Felix test were calculated using the DS test as the "gold standard".

Results: Taking the DS as the "gold standard", at a cutoff titer of 1:160 (> 1:160 is considered antibody detected), the Weil Felix had a sensitivity of 27% and specificity of 99.5% for *R. typhi*. Positive and negative predictive values were 95.2% and 79.2% respectively. The number of positives cases for *R. rickettsia* and *O. tsutsugamushi* (Karp strain), and *O. tsutsugamushi* (Kato and Gilliam strains) were too low to calculate the sensitivity and specificity accurately.

Conclusion: Though costly, the greater sensitivity, shorter turnaround time and simplicity of the DS compared to the Weil Felix suggest that the DS is a satisfactory replacement test for the Weil Felix for routine testing for rickettsial infections.

I119/PD

Evaluation of the Clinical Impact Through a Focused Drug Therapy Review Programme in In-flight Patients with Renal ImpairmentSok Bee LIM¹, Gek Kee LIM¹, Ai Leng KHOO¹, Pary SIVARAMAN²¹Department of Pharmacy, National University Hospital, Singapore, ²Department of Medicine, National University Hospital, Singapore**Aim:** To evaluate the clinical impact of pharmacists' involvement in the drug therapy review of chronic renal failure (CRF) and end-stage renal disease (ESRD) patients.**Methods:** A detailed medication review was conducted for all CRF or ESRD patient admitted to the renal ward in NUH over a 2-month period. Prospective therapeutic monitoring together with concurrent feedback to physicians was carried out during daily ward round. In addition, recommendations, other than those to resolve an identified drug-related problem (DRP), that could potentially improve a patient's pharmacological management were also proposed. Each DRP and recommendation was assigned a significance rank based on a published scale by a renal physician. The scale comprises 6 levels of significance, ranging from 1 (adverse significance) to 6 (extreme significance).**Results:** Sixty cases were recruited into the study. Eighty-six DRPs (with acceptance rate of 93%) and 10 recommendations (with acceptance rate of 90%) were recorded. Transcription error made up 44% of the 86 DRPs while renal dosage adjustment accounted for approximately 10%. The main DRPs involved renal (27%), anti-infective (17%) and cardiovascular medications (16%). 70% of the recommendations were laboratory investigations. The significance ranking of the reported DRPs were 67% as level 4, 26% as level 3, 4% as level 5 and 4% as level 2. There was no DRP with a significance ranking of level 1 or 6.**Conclusion:** Pharmacists can play a significant role in the identification of potential or actual DRPs and through a concerted effort with renal physicians, optimize the pharmaceutical care of renal patients.

I120/PD

Stability of Spironolactone Suspension Using 1% and 3% Methylcellulose as the Suspending Agent

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Aim: Spironolactone is available commercially as tablets but not in suspension form. The problems with extemporaneously prepared syrup are settling of drug particles, which can lead to non-uniform drug dosing and short expiry. The objectives of this study were to reduce the caking problem of the current spironolactone suspension and to extend the expiry date of the current spironolactone suspension by using varying concentration of the suspension agent, methylcellulose.**Methods:** Spironolactone suspension was prepared with tablets suspended with methylcellulose (MC) 1% and 3%. 10 bottles each of 1% and 3% methylcellulose as the vehicle were prepared. Stability testing using HPLC was done at Day 0 and on Day 85. Suspendability and re-suspendability tests were also conducted to assess the caking problems of the syrup.**Results:** Results indicated no significant change over time (85 days) for the suspension with 1% methylcellulose with P value 0.152. However, there is a statistical significance for the preparation with 3% methylcellulose (P = 0.001) when it is stored for 85 days.**Conclusion:** Spironolactone suspension in 1% MC is stable for up to 12 weeks when stored at 4°C. The suspendability and re-suspendability results show no advantage of 3% MC over 1% MC in the reduction of caking of these suspension.

I121/PD

The Relationship Between Isokinetic Quadriceps Muscles Parameters and Countermovement Jump in Active Healthy Females: An Allometric Scaling ApproachYong Hao PUA¹, Yik Ying TEO², Michael Teik Hin KOH³¹Rehabilitation, Alexandra Hospital, Singapore, ²Defence Medical Research Institute, Defence Science and Technology Agency, Singapore, ³Physical Education and Sports Science, National Institute of Education, Singapore**Aim:** The utility of isokinetic quadriceps measurements in sports rehabilitation

is dependent on its magnitude of correlation with functional performance measurements. Previous attempts to correlate both measurements have, however, produced inconsistent results. Consequently, opinions differ regarding the isokinetic parameters employed during rehabilitation. Allometric scaling, to adjust for body mass differences, is used increasingly in exercise sciences, and is recently proposed to provide the strongest possible relationship with jump measurements. The purpose of our study is to use allometric modelling to examine the 1) isokinetic parameter that best correlates with countermovement jump (CMJ) and 2) strength-performance relationship.

Methods: Twelve non-elite females (age = 21.0 ± 2.0 years, body mass index = 20 ± 1.5 kgm⁻²) performed isokinetic quadriceps and CMJ testing. Allometric-scaled units were derived from a log-log transformation of body mass, and isokinetic peak torque and average power obtained reciprocally and non-reciprocally at 1.04 rad·s⁻¹ and 3.14 rad·s⁻¹. Coefficients of determination (r²) were calculated to determine the magnitude of relationships between isokinetic and CMJ measurements.**Results:** r² ranged from 0.06 to 0.13 and 0.29 to 0.37 for reciprocal and non-reciprocal isokinetic parameters, respectively. When non-reciprocal isokinetic parameters were allometrically corrected for body mass, 2.6–21.8% of the CMJ height variance was additionally accounted for. Specifically, isokinetic average power at 1.04rad·s⁻¹ had the highest correlation with CMJ (r² = 0.59).**Conclusion:** The ability to produce power is a significant criterion in CMJ. The use of isokinetic non-reciprocal average power as an indicator of muscle performance is based on biological and physical/Newtonian principles, and is supported empirically by our findings. Isokinetic parameters should be allometrically scaled to reveal correlations with CMJ that may otherwise remain obscure.

I122/PD

Error Estimates of the KT2000 ArthrometerYong Hao PUA¹, Peck Hoon ONG²¹Rehabilitation, Alexandra Hospital, Singapore, ²Rehabilitative Services, Alexandra Hospital, Singapore**Aim:** The KT2000 arthrometer is commonly used in the diagnosis of anterior cruciate ligament (ACL) deficiency and to document the state of recovery, both required datum involving test-retest statistics. Previous studies have calculated the standard error of measurement (SEM), but did not extrapolate the measurement error to analytical goals in the clinical setting. The purpose of our study is to examine the reliability of KT2000 measurements in clinical applications**Methods:** A single-group repeated measures design was used. Two testers performed measurements on 72 knees of 36 subjects (age = 22 ± 4 years) with suspected unilateral ACL insufficiency. Measurements were recorded at 131N. Relative reliability is expressed in terms of intra-class correlation coefficients (ICC formula [3,1]). Absolute reliability is expressed in terms of the smallest detectable difference (SDD) index.**Results:** The inter-tester ICCs were 0.86 and 0.68 for the involved and uninvolved side, respectively. The SDDs (95% confidence interval [CI]) were 3.75 mm and 2.67 mm for the involved and uninvolved side respectively. The overall inter-rater error estimate was 3.24 mm.**Conclusion:** Measurements from the involved side were more useful for discriminating amongst patients, whereas the magnitude of error is less for measurements from the uninvolved side. The SEM represents the error associated with a single measurement while the SDD provides a reference interval for a test-retest difference on a 95% CI basis; the latter may be a clinically more appropriate index. When considering that the overall inter-rater error exceeds the empirical threshold value of 3.0 mm for diagnostic purpose, we recommend that repeated measurements should be undertaken by the same examiner whenever possible.

I123/PD

Allometric Scaling of Isometric Knee Extensor Peak Torque in Male Subjects with Unilateral Knee InjuriesYong Hao PUA¹, Yik Ying TEO²¹Rehabilitation, Alexandra Hospital, Singapore, ²Defence Medical Research Institute, Defence Science and Technology Agency, Singapore**Aim:** Muscular strength (S) is associated with body mass (M) with $S = k \times M^b$, based on allometric modelling. Although controversy continues concerning the value of the exponential parameter, *b*, the qualitative difference

between S , defined as force, and torque has often been ignored. Because isometric knee extensor peak torque is frequently measured during rehabilitation, the purpose of our study was to use allometric scaling to examine the influence of body mass on torque measurements.

Methods: Using an isokinetic dynamometer, isometric knee extensor peak torque at 65° flexion of the uninvolved side was recorded in 116 males with unilateral knee injuries. To ensure greater homogeneity of body composition in the sample, an analysis of the coefficient of variation for torque measurements with increasing body mass index (BMI) yielded a threshold of minimal variability at 22.9 kgm⁻². Consequently, allometry was performed separately on torque measurements obtained from subjects with BMI of 22.9 kgm⁻² (49 males, age = 22.6 ± 4.1 y, M = 62.3 ± 6.2 kg, BMI = 20.8 ± 1.6 kgm⁻²) and BMI >22.9 kgm⁻² (67 males, age = 23.2 ± 2.8 y, M = 77.2 ± 9.9 kg, BMI = 25.8 ± 2.7 kgm⁻²).

Results: Allometric scaling exponents (95% confidence interval [CI]) were 0.93 (0.6-1.3) and 1.1 (0.71, 1.50) for the BMI of 22.9 kgm⁻² and BMI >22.9 kgm⁻² groups, respectively.

Conclusion: Both mass exponents were closer to 1 than 0.67, and 0.67 was excluded in the 95% CI in the BMI >22.9 kgm⁻² group, which supported the dimensionality theory that predicts $b = 0.67$ and 1 for force and torque measurements, respectively. Our findings suggest that ratio standards method ($b = 1$) may be used to adjust isometric knee extensor torque measurements for body mass differences to allow for inter-subject comparison.

I124/PD

Correlation of Knee Stability to Functional Outcomes after Anterior Cruciate Ligament Reconstruction: A Cross-sectional Study

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Aim: Traditionally, evaluation of outcomes after an anterior cruciate ligament (ACL) reconstruction has been limited to knee strength testing, clinical knee laxity test, knee range of motion and subjective reports. However, there is a recent trend towards the use of functional testing procedures, such as hop tests, that are time-efficient, economical and easy to administer. This study aims to investigate the relationship between knee laxity test, isokinetic knee strength, a self-administered Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire and 2 hop tests, single leg and crossover hop. The feasibility of using the 2 hop tests and KOOS questionnaire as alternative outcome measurements post-ACL reconstruction are also assessed.

Methods: 45 subjects were recruited for this cross sectional study. The KOOS questionnaire, KT2000 knee arthrometer, isokinetic knee strength and 2 hop tests were administered to them.

Results: Results showed that both hop tests were highly correlated ($r = 0.75$) to each other. They had moderate to good correlation ($r = 0.47$ - 0.62) to peak torque quadriceps muscles, fair correlation ($r = 0.26$ - 0.37) to peak torque hamstrings muscles and KOOS questionnaire, and little correlation ($r = 0.02$) to KT2000. KOOS questionnaire had fair correlation ($r = 0.25$ - 0.43) to both peak torque quadriceps and hamstrings muscles, and little correlation ($r = 0.19$) to KT2000.

Conclusion: These results indicate that hop tests may only reflect strength of quadriceps muscles. The relatively low correlation between the different outcome measures may indicate that neither of these measures can stand alone as an adequate assessment.

I125/PD

Application of Generalised Estimating Equations in Longitudinal Studies

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Aim: In a longitudinal study, the researcher follows a cohort of subjects over time, and performs repeated measurements at prescribed intervals. The measurements are correlated and, hence, violate the iid assumption upon which many likelihood statistical models are based. The aim of this paper is to demonstrate the appropriateness and usefulness of generalised estimating equations (GEEs) in analysing data obtained from longitudinal studies.

Methods: A Monte Carlo experiment was conducted to examine the properties of GEE models. To illustrate, 4 correlated measurements – the first as baseline and the rest as repeated data made at 3 different visits – were generated from a multivariate normal distribution with sample size fixed at 1000. The repeated data follow an autoregressive correlation structure with $\rho = 0.8$. Several GEE models with different correlation structures, namely, AR1, AR2, exchangeable and independent, were applied to analyse the data. All statistical tests were carried out at 5% level of significance. The data were analysed with Stata 7.0.

Results: The appropriate GEE models showed that conventional regression methods underestimate between-subject factors (such as, baseline measurements and treatment covariates) but overestimate within-subject factors (such as visits). If GEEs were not applied, one might arrive at incorrect decisions concerning the coefficients of the regression model.

Conclusion: Treating repeated observations obtained from longitudinal studies as independent is unrealistic and will certainly lead to poor estimates of the standard errors. As such, GEE models are advocated for analysing longitudinal data.

I126/PD

Expressed Emotion in Singapore: A Qualitative Enquiry

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Aim: The aim of this study was to investigate the concept of Expressed Emotion (EE) and the applicability of the Level of Expressed Emotion (LEE) scale in Singapore.

Methods: This was a qualitative study. Regarding EE, 10 patient-caregiver pairs were recruited from an ongoing study of patients experiencing their first episode of psychosis and interviewed using a semi-structured interview format. Regarding the LEE, small focus-group interviews were conducted with a cross-section of Singaporeans to gain their views on the concepts in the scale and the relevance of the items in this culture.

Results: For the first part, the data were analysed in light of attitudes found to discriminate between high and low EE families. A clear distinction between high and low EE groups was found. For the second part, the data from the focus groups supported the cross-cultural conceptual and operational equivalence of the scale. During discussion, several additional emic items were suggested by the participants.

Conclusion: There is support for the existence of EE in Singapore and the LEE was found, for the most part, to be applicable in Singapore, with the addition of several emic items.

I127/PD

Development of a Prostate Specific Antigen Transfected Murine Bladder Cancer Cell Line as a Means of Evaluating Gene Therapy for the Treatment of Bladder Cancer Using an Orthotopic Murine Model

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Aim: Our aim is to develop a simple, reliable and non-invasive method to monitor bladder tumour growth in a murine model during treatment. We will use this technique to evaluate the treatment effect of cytokine gene therapy on bladder cancer.

Methods: Murine bladder cancer cell line MB49 was permanently transfected with a plasmid vector pSecTag2/PSA so that it will produce PSA as a marker. After antibiotic selection, the resistant cells were cloned by limiting dilution. The clones were then screened for PSA production by sandwich ELISA assay. An orthotopic murine bladder cancer model was established by intravesical implantation of the modified MB49 cells. Then the serum and urine PSA levels were measured as indicators of tumour growth. The PSA level was also continuously measured following intravesical gene therapy using Granulocyte Macrophage Colony Stimulating factor (GM-CSF).

Results: The modified cells were shown to be tumourigenic and produced high level of PSA both *in vitro* and *in vivo*, detectable by ELISA. After intravesical implantation of the tumour, the PSA could be detected both in mouse urine

and serum from day 4 onwards. The level of PSA produced increased progressively and correlate with the tumour volume. The PSA level dropped following intravesical gene therapy using GM-CSF. Tumour regression was also confirmed by histology.

Conclusion: This study has provided a simple, non-invasive method to monitor orthotopic bladder tumour growth and the results confirmed that cytokine gene therapy is effective in the treatment of bladder cancer.

I128/PD

Distance and Velocity Requirements for Functional Community Ambulation in Singapore

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Aim: We document the distances and velocities that individuals must ambulate to function independently in the community.

Methods: Eighteen HDB blocks from 3 housing estates of different age categories (old, mature and new) were randomly selected. Distances between these blocks and the nearest amenities (such as, bus-stops, taxi stands/main roads, coffee shops, grocery shops, clinics, senior citizen centres and ATM machines) were measured. Distances across the roads in these estates and the time allowed by signals for safe crossing were also measured. From these data, the velocities needed to cross the roads safely were calculated.

Results: The results showed that the distances between the blocks and the nearest amenities were shortest in the old estates, followed by the mature estates, and the longest in the new estates. Individuals living in the old, mature and new estates will have to travel for an average distance of 254 m (range 67-834), 288 m (range 71-870) and 470 m (range 81-812), respectively, to the nearest amenities. The average velocities required to cross a road safely in the old, mature and new estates were 0.68 m/s, 0.81 m/s and 0.79 m/s, respectively.

Conclusion: Based on the results of this study, therapists will be able to set realistic community ambulatory goals for patients undergoing rehabilitation.

I129/SOGD

Risk Factors for Postoperative Renal Dysfunction after Deep Hypothermic Circulatory Arrest for Repair of Thoracic Aortic Aneurysm and Dissection

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Aim: The use of deep hypothermic circulatory arrest (DHCA) in cardiovascular surgery has been identified as a predictor of acute renal failure and contributes to significant morbidity and mortality. Aim: We aim to determine the incidence of and to elucidate the risk factors for renal dysfunction after DHCA for repair of thoracic aortic aneurysm and dissection.

Methods: From the cardiothoracic databases of National University Hospital and National Heart Centre, 59 patients were identified to have undergone repair of thoracic aortic aneurysm and dissection from January 1999 to April 2002. Chart review was performed to extract data pertaining to medical history, operative details and postoperative progress. Postoperative renal dysfunction was defined as a rise of serum creatinine $>177 \mu\text{mol/l}$ for patients with normal preoperative creatinine, doubling of creatinine from preoperative values for patients with pre-existing impaired renal function or requirement for postoperative renal dialysis.

Results: 36 (61%) of the 59 patients sustained postoperative renal dysfunction. 11 (30.6%) of these 36 patients required postoperative dialysis. 7 (63.6%) of these 11 patients died perioperatively. Significant risk factors for renal dysfunction were elderly age, longer bypass time, lower haematocrit and base excess during bypass, more bicarbonate administration and usage of blood salvage devices. There was a trend towards longer DHCA and aortic cross-clamp times, although this did not reach statistical significance.

Conclusion: Older age, longer bypass time, lower haematocrit and presence

of acidosis during bypass were identified as risk factors for renal dysfunction after DHCA for repair of thoracic aortic aneurysm and dissection.

I130/SOGD

Faster Resumption of Spontaneous Respiration after LMA Insertion Using Low-dose Fentanyl in Local Population

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Aim: Fentanyl is an adjuvant commonly used with propofol to facilitate insertion of the LMA. This is often followed by a variable period of apnoea that can sometimes be prolonged. Some patients may benefit from faster resumption of spontaneous breathing, including day surgery cases or procedures of short duration. The purpose of this study was to investigate if a clinically useful reduction in apnoea can be achieved using a lower dose of fentanyl, and how it would affect the conditions for LMA insertion.

Methods: 124 ASA I or II patients aged 16-65 years requiring minor surgery were included. They were randomised to receive either fentanyl 0.5 mcgkg^{-1} (F0.5) or fentanyl 1.0 mcgkg^{-1} (F1). This is followed by propofol 2.5 mgkg^{-1} . LMA insertion was attempted 90 seconds later. The time taken for patient to resume spontaneous respiration was noted. The conditions for insertion (determined by a 3-point scale on 5 variables) and changes in blood pressure, heart rate and pulse oximetry were recorded.

Results: Both groups were demographically similar. Apnoea was shortened in Group F0.5; at 30 seconds after insertion of LMA, 78% of patients had started breathing spontaneously compared to 41% of patients from Group F1 ($p < 0.001$). Conditions for insertion were not significantly different between both groups.

Conclusion: A lower dose of fentanyl can be used in our local population during LMA insertion without significantly affecting the conditions for insertion if faster resumption of spontaneous respiration is desired.

I131/SOGD

Excessive Hydrostatic Distension Reduces Vasoreactivity of Radial Artery Conduits

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Aim: Surgical preparation of coronary conduits may affect early and long-term patency through endothelial and smooth muscle injury. We investigated the effect of hydrostatic distension on in-vitro endothelial function and contractile properties of human radial artery (RA).

Methods: Human RAs were harvested and distended at physiological or supra-systemic pressure ($>300 \text{ mm Hg}$) for 2 minutes. Distal segments were retrieved and cut into 3-mm rings which were mounted and equilibrated in oxygenated Krebs's solution at 37°C . Contraction responses to potassium chloride (KCl), noradrenaline and serotonin, and relaxation responses to acetylcholine (ACh) and nitroprusside, were evaluated. Undistended rings were used as controls.

Results: Contraction to all 3 contractile agonists were significantly different between the groups, with supra-systemic pressure distension achieving the lowest percentage of maximum contraction (KCl $p < 0.001$; noradrenaline $p < 0.05$; serotonin $p < 0.05$). EC50 was also significantly reduced, indicating increased sensitivity to all 3 agonists. Only receptor-mediated contractility was significantly reduced in the physiologically distended group when compared to controls. Relaxation to ACh and nitroprusside was significantly reduced in the supra-systemic pressure distended group, with a tendency to vasospasm when exposed to a physiological concentration of ACh (10^{-6} M). EC50 to both ACh and nitroprusside were not different between the groups.

Conclusion: Excessive distension of RA leads to a significant reduction in vasoreactivity, which may be attributed to a disruption of the vascular endothelium and media with a propensity for graft spasm when exposed to ACh.

1132/SOGD

Risk Scoring Systems for Coronary Artery Bypass Graft (CABG): Do They Apply to Asian Patients?

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Aim: Risk scores need to be applied in comparing outcomes of coronary artery bypass graft (CABG). However, various scoring systems are based on different designs and patient populations. Commonly used risk stratification systems are derived from North American and European populations. They have not been evaluated for applicability to Asian populations. This study aims to address the issue.

Methods: Between 1 January 2000 and 31 December 2002, data of 1054 consecutive patients undergoing isolated primary CABG surgery in our institution was prospectively scored and collected by an independent observer. Mortality predictions were prepared using the Parsonnet, Euroscore and Northern New England systems. Postoperative 30-day mortality was registered. Score validity was assessed by the area under the receiver operating characteristic (ROC) curve. Odds ratios were calculated to investigate the predictive relevance of risk factors.

Results: The predicted mortality for our population was 6.91 + 7% by Parsonnet, 4.4 + 3% by Euroscore, and 1.7 + 1% by the Northern New England scoring system. Observed mortality at this centre was 2.8%. The area under the ROC curves were Parsonnet 0.8, Euroscore 0.87, and Northern New England 0.841. Most surgeons outperformed Parsonnet and Euroscore.

Conclusion: The risk scores derived from a predominantly Caucasian population are applicable to our patients. Euroscore had the highest predictive value in our total patient population.

1133/SOGD

Achievement of Technical Satisfaction after Proximal Aortic Anastomosis Using CorLink Aortic Anastomotic Device with Intraoperative Flowmeter Study in Isolated Coronary Artery Bypass Graft (CABG)

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Aim: We demonstrate equivalency of the CorLink aortic anastomotic (AAD) anastomoses compared to hand-sutured anastomoses in patients undergoing coronary artery bypass graft (CABG) with respect to 6 months patency at proximal anastomotic site.

Methods: Medi Stern flowmeter was used in all patients as standard method of assessing graft patency intraoperatively in our study. A total of 25 patients undergoing isolated CABG were randomised to using AAD (13 patients) and conventional hand suture technique (12 patients). Two patients assigned to AAD were converted to hand suture technique. A total of 16 AADs were used in 13 patients in the device group. All patients were reviewed at the first, third and sixth month after the operation. The patency of proximal anastomosis was evaluated using high speed computed tomography scan at follow-up 6 months later.

Results: The mean time of application of the device was 2 minutes and the mean flow rates using AAD and the hand suture technique were 24.68 ± 16.18 l/min and 30.50 ± 20.83 l/min, respectively. Pulsatility index in the flowmeter was 2.82 ± 1.61 for the hand suture technique and 5.65 ± 5.6 for the AAD group. There was no significance difference in the occlusion rate between the 2 techniques. In our study, the percentage of occlusion was 37.5% and 15.8% for AAD and the hand suture technique, respectively (p = 0.143). This was comparable with the overall result (p = 0.610) from combined groups (5 centres), which was 26.1% and 22.5% for AAD and hand suture technique, respectively.

Conclusion: Corlink AAD is safe, effective and efficient in creating proximal aorto-saphenous anastomosis besides the advantage of avoiding side clamping of the aorta.

1134/SOGD

Comparison of Tooth Slice and Immortalised Cell-Line for Cytotoxicity Testing

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Aim: Organ culture consists of dental cells organised *in vivo*, which is highly relevant to cytotoxicity testing of appropriate dental products. Immortalised cell-line cultures, on the other hand, are simple replicating systems without any specific metabolic potential that the target cells have *in vivo*. In this study, organ culture cytotoxicity testing was compared to that of immortalised cell-line to determine the sensitivity and reproducibility of both culture models.

Methods: For the organ culture, a 28-day old incisor, sliced into a thickness of 2 mm, was cultured with and without agarose culture gel and submerged in complex media. Direct contact test involved the restorative dental composite to come into contact with the exposed pulp-dentin complex. Indirect contact involved the composite contacting the agar barrier between the incisor slice. Differences in morphological intact cell numbers were assessed. For cell-line culture, L-929 (ISO-approved and highly recommended) was used. Direct test involved the monolayer cells to contact the composites, whereas the indirect test had an agar barrier. Zone of inhibitions was assessed. All results were analysed using Tukey one-way ANOVA (p<0.05).

Results: Direct contact organ cytotoxicity tests were more sensitive than indirect ones as more dental composites were toxic compared with controls. All test composites were non-toxic from L-929 direct contact test (p>0.05) and were significantly toxic from the indirect contact tests (p<0.001). Cytotoxicity rankings of the dental composites were similar for both culture models.

Conclusion: L-929 cytotoxicity tests are extremely sensitive, giving compatibility of the materials for 1 test and absolute toxicity for the other. Organ culture tests provided more explainable results; direct tests were more sensitive than indirect tests with the agar barrier, which reduced the response of toxic elements.

1135/SOGD

A Comparison of Hyperbaric Solutions of Levobupivacaine and Racemic Bupivacaine Administered Intrathecally with Fentanyl for Caesarean Delivery

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Aim: Levobupivacaine is the pure S(-) enantiomer of racemic bupivacaine with reduced cardiovascular and central nervous system toxicity. In this prospective, randomised and double-blinded study, we compare the clinical efficacy, characteristics and side effects of intrathecal levobupivacaine with that of racemic bupivacaine for elective caesarean delivery. Fentanyl was added to improve the quality and duration of anaesthesia.

Methods: 41 full-term singleton parturients were recruited. Patients received intrathecal injection of fentanyl 10 mcg, with 9 mg of either 0.5% hyperbaric levobupivacaine or 0.5% hyperbaric racemic bupivacaine. Assessments of sensory block to pin prick and motor block (using a modified Bromage scale) were performed in addition to routine monitoring. Evaluation of side effects and patient satisfaction scores were performed postoperatively.

Results: The onset of motor block in the levobupivacaine group was significantly slower (mean, 7.3 ± 2.6 vs. 4.9 ± 2.6 sec, P < 0.01), with a greater proportion of patients not achieving Bromage 3 motor block (24% vs. 0%, P < 0.01) compared to the racemic bupivacaine group. Furthermore, regression of motor block to T10 dermatome was significantly faster in the levobupivacaine group (mean, 89 ± 26 vs. 148 ± 26 min, P < 0.01). Other spinal block characteristics, incidence of side effects and patient satisfaction scores were similar in both groups.

Conclusion: Intrathecal levobupivacaine with fentanyl for elective caesarean section produced effective spinal anaesthesia, but with a shorter duration and

lesser degree of motor block compared to racemic bupivacaine. The faster recovery of motor function allows earlier ambulation, which will theoretically decrease the risk of deep vein thrombosis.

I136/SOGD

Comparison of Clinical with Pathological Nodal Status from Systematic Mediastinal Lymph Node Dissection in Surgery for cN0 Non-small Cell Lung Cancer

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Aim: We compared the accuracy of clinical nodal (cN) status N0 with that of pathological nodal (pN) status obtained from systematic mediastinal lymph node dissection (SMLD) in primary non-small cell lung cancer (NSCLC).

Methods: Data from 22 consecutive patients who underwent lung resection and SMLD, of at least 3 mediastinal node stations, from November 2001 to May 2003 were analysed retrospectively. Only patients with cN0 status on computed tomography (CT) scan referred for curative surgery were included in this study.

Results: The mean age of the patients was 66.6 ± 8.1 years and the male to female ratio was 17:5. The mean number of lymph node stations dissected was 5.8 ± 1.8 . Forty percent (9/22) had squamous cell carcinoma, 45% (10/22) had adenocarcinoma, 5% each had large cell carcinoma, bronchioalveolar carcinoma or a lympho-epithelial carcinoma. pN2 metastases were found in 27% (6/22) of patients. The sensitivity of cT was 70%, with a specificity of 50%. For cN, the sensitivity was only 7.1% with a specificity of 97.1%. The positive and negative predictive values of cN status were 50% and 71.1%, respectively. Curative resections were achieved in 59% (13/22) of patients.

Conclusion: The sensitivity of cN0 status, based on CT scan alone, is extremely poor when compared with pN status from SMLD. In our analysis, based on cN status alone, 27% of patients in pN2 status would have been understaged. We recommend that all patients with cN0 status should undergo SMLD, of at least 3 appropriate mediastinal node stations for proper staging.

I137/SOGD

Doppler Detection of Arterio-arterial Anastomosis Predicts Survival Outcome Independent of Quintero Stage in TTTS

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Aim: We seek to determine if the antenatal detection of arterio-arterial anastomoses (AAA) predicts survival independently of Quintero stage in twin-twin transfusion syndrome (TTTS).

Methods: One hundred and seven consecutive cases of TTTS were studied between 1995 and 2003. AAA and Quintero stage were determined at presentation and at each subsequent visit. The main outcome measures were rates of perinatal survival, double survival and any survival.

Results: Ninety-six pregnancies were analysed. The overall rates of perinatal survival, double survival and any survival were 60%, 44% and 76%, respectively. Survival was better when an AAA was detected at presentation than when it was not in terms of perinatal survival rates (78% vs 56%, $p = 0.02$) and double survival rates (72% vs 37%, $p = 0.007$). The differences in rates widened with increased AAA detection rates at treatment and before delivery. The outcome was poorer with increased Quintero stage at presentation in terms of perinatal survival rates (74%, 63%, 50% and 45%, $p = 0.001$) and double survival rates (66%, 50%, 33% and 0%, $p < 0.001$). Both AAA and stage were demonstrated to be independent predictors of perinatal survival rates and double survival rates by multiple logistic regression analysis. The presence of antenatally detected AAAs conferred better outcomes for Quintero stages I to III before the first invasive treatment in terms of perinatal survival rates and double survival rates.

Conclusion: Antenatal detection of AAA predicts better survival outcomes independent of Quintero stage. Its incorporation into a modified staging system improves prognostication.

I138/SOGD

Standardised MR Classification System for Reporting of Degenerate Lumbar Discs: Interobserver Variability and Implications

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Aim: A classification system has been proposed for the reporting of MR lumbar disc contour abnormality. We evaluate the system for interobserver variability and implications of using this system for routine MR reporting of lumbar spine studies.

Methods: 100 consecutive cases of MR lumbar spine studies were evaluated by 2 radiologists using the new classification system jointly proposed by the North American Spine Society, American Society of Neuroradiology and American Society of Skeletal Radiology. Disc contours were classified as: normal, symmetrical disc bulge, asymmetrical disc bulge, focal protrusion, broad-based protrusion and extrusion. Statistical analysis was performed for each category for percentage agreement.

Results: Interobserver agreement among all categories is 83%. Kappa value yielded 0.8, corresponding to substantial agreement.

Conclusion: Using the new proposed system, substantial agreement was achieved between observers, making the new classification a robust system. Some difficulties and pitfalls in using this new system include the effects of osteophytes on disc contour, incongruity of L5 vertebral body on S1 and the differentiation from the normal, but slightly bulging, L5 disc.

I139/SOGD

Extension of the Posterior Interosseous Reverse Flow Flap

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Aim: The posterior interosseous reverse flow (PIRF) flap is a well-established and robust option for the coverage of skin and soft tissue defects of the hand. It provides excellent tissue matching for the hand and does not require microsurgical expertise. It is commonly used over the region of the hand dorsum and first web. We have been able to extend the flap to cover defects more distally over the thumb and fingers, up to the level of the distal interphalangeal joint. We present our experience using the modified version of this flap.

Methods: Three cases of hand injuries affecting the thumb or fingers were reconstructed using an extended PIRF flap. Each case was discussed, emphasising the various reconstructive options and our rationale for using this flap. The technique used was discussed, particularly the need to include as many perforators as possible with a large skin paddle and de-epithelialisation of the tunnelled portion of the flap.

Results: In the 3 clinical cases presented, the flap was used successfully to cover defects in the thumb or fingers of the hand. Modification of the flap allows a further reach than the original flap design.

Conclusion: The PIRF flap is robust and versatile. Methods to extend the reach and reliability of the flap have been reported. The application of a breast reconstruction technique by taking a larger/longer flap than necessary to include more perforators, and de-epithelialising the tunnelled portion have allowed us to achieve extended reach up to the level of the distal interphalangeal joint of the fingers. This technique can also increase the reliability of the flap.

I140/SOGD

Management and Outcome of Adult Blunt Splenic Trauma

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Aim: The spleen is the most commonly injured organ in blunt abdominal trauma. Preservation is often advocated in view of the spleen's immunological importance. The aims of this study were to describe management and

outcome among adult patients with blunt splenic trauma seen at our hospital, and to determine the rate of splenic salvage.

Methods: A retrospective review of adult patients treated for blunt splenic trauma at our hospital between January 1994 and June 1997 was conducted.

Results: 34 patients with documented blunt splenic trauma were identified. Overall, 22 (65%) were definitively managed by splenectomy, 8 (24%) by splenorrhaphy and 3 (9%) by non-operative management. One had his splenic injury left alone at laparotomy as it was not actively bleeding. The mortality rate was 18%. The splenic salvage rate was 35%.

Conclusion: Splenectomy is the commonest method of management for adult blunt splenic trauma and could reflect the greater familiarity with this modality among our surgical staff. Splenic preservation is achieved in 35% of patients, mostly by splenorrhaphy. The use of a standardised management protocol may improve our salvage rate.

I141/SOGD

Soft Tissue Injury Patterns in Motorcycle Accidents

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Aim: A prospective study was conducted to describe the pattern of distribution and possible contributing factors of soft tissue injuries sustained by motorcyclists admitted to a hospital in Singapore. This information would provide insight into the likely cause and effect of soft tissue trauma in such accidents, as well as to provide a basis for work on improvement of existing safety measures.

Methods: Twenty-three inpatient motorcyclist casualties were studied over a period of 3 months. The circumstances of their accidents and the distribution and nature of their injuries were described. On follow-up, the duration of medical leave taken for their injuries was recorded.

Results: Injuries to the face (69.6%) and upper limbs (91.3%), in particular the dorsum of the hands (65.2%), were most commonly seen, the majority being superficial abrasions of varying surface area. While the distribution of injuries was largely random, there was a correlation between severity of injury with speed of travel and use of personal protective equipment. There was no clear relationship observed between the distribution of soft tissue injuries and bony or visceral injuries. Injuries resulted in patients being given, on average, 1 month's leave from work.

Conclusion: Despite existing safety measures, motorcyclists' faces and hands remain particularly vulnerable to soft tissue injuries. These injuries contribute to a significant part of annual medical leave.

I142/SOGD

Theophylline May Have a Role in the Management of Ureteric Colic

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Aim: We study the side effects and efficacy of orally administered theophylline in a double-blind, randomised phase II clinical trial in patients with ureteric colic.

Methods: Fifty-three patients with proven, uncomplicated single ureteric calculi 10mm or less in size were randomised to receive either theophylline or an identical placebo twice daily for a period of 8 weeks. 19 patients were on placebo and 34 patients were on theophylline. Each patient received a standard analgesia regime and a symptom diary during the 8-week period to document symptoms and side effects of therapy. Stone passage was documented with plain abdominal radiographs 4 and 8 weeks following therapy.

Results: Twelve patients on theophylline (n = 34) passed their calculi within 8 weeks, of which 66.7% of them did so within the first 4 weeks. Eight patients on placebo (n = 19) passed their calculi within 8 weeks, of whom 62.5% did so in the first 4 weeks. Both pain intensity and analgesic consumption were less in the theophylline arm. Side effects, such as vomiting,

palpitations and gastric discomfort, were minor and transient.

Conclusion: Theophylline may be a useful adjunct to standard analgesic medication in decreasing the intensity of ureteric colic and reducing analgesic use. This may reduce visits to the emergency room for pain relief.

I143/SOGD

Virus-mediated Small Interfering RNA Directed Against the Laminin-5 γ 2 Chain Reduces Secretion of Laminin-5, but Increases Tumour Cell Proliferation and Migration in Squamous Cell Carcinoma

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Aim: Laminins are heterotrimeric basement membrane glycoproteins. In squamous cell carcinoma (SCC), Ln-5 is overexpressed, especially at the invasive front. To understand the role of Ln-5 in SCC motility and invasion, we used small interfering RNAs (siRNAs) to inhibit the expression of Ln-5 in SCC cells.

Methods: UM-SCC-22B (22B), a highly invasive human SCC line, does not produce Ln-5. In contrast, JHU-022-SCC (022) is non-invasive, but secretes Ln-5. siRNA oligonucleotides directed against the Ln-5 γ 2 chain were cloned into a retroviral expression vector and transfected into amphotrophic packaging cells. Viruses expressing the siRNAs were used to infect 022 cells (022-si γ 2) and were selected by resistance to puromycin.

Results: Expression levels of the Ln-5 α 3 and β 3 subunits remained unchanged in both parental 022 and 022-si γ 2 cells. In contrast, expression of Ln-5 γ 2 chain was nearly undetectable in lysates and media conditioned by the 022-si γ 2 cells compared to parental cells. Furthermore, the levels of α 3 and β 3 chains were reduced in media of 022-si γ 2. 022-si γ 2 cells grew 2-fold faster and were 5-fold less resistant to trypsinisation compared to parental 022 cells. Finally, 022 cells migrate poorly on matrices whereas 022-si γ 2 cells migrated nearly as efficiently as 22B cells. The enhanced migration of 022-si γ 2 cells could be blocked by antibodies to Ln-5 and Ln-5 receptors.

Conclusion: The Ln-5 γ 2 chain regulates the secretion of the α 3 and β 3 subunits and is important in the secretion of the Ln-5 heterotrimer. Loss of γ 2 expression decreased cellular adhesion while increasing SCC proliferation and migration to levels similar to invasive SCC cells.

I144/SOGD

Total Knee Arthroplasty in the Severely Deformed Neuropathic Joint

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Aim: Severe progressive neuropathic arthropathy of the knee joint is uncommon. Surgical treatment has traditionally been arthrodesis. Recent reports with medium-term follow-up suggest that total knee arthroplasty (TKA) may be a viable option in these patients, avoiding fusion. We report early results of a series of 6 patients (12 knees) with severely deformed neuropathic knees for which TKA was performed.

Methods: We retrospectively analysed the records of patients with severely deformed neuropathic knee arthropathy who had TKA performed by the same surgeon. Cemented long-stem TKA prostheses with bone grafting and augmentation were used in all patients. Bilateral TKA, when required, was performed in a single operation. Patients were assessed at follow-up using the Hospital for Special Surgery Knee Disability Sheet for Total Knee Arthroplasty (HSS) and a visual analogue scale for pre-operative and post-operative pain, mobility and quality of life.

Results: The mean follow-up of the patients was 15 months (3-26 months). All patients were alive at follow-up. There were no perioperative complications. The mean HSS score at follow-up was 90 points. VAS scores for pain,

mobility and quality of life before and after the operation improved by means of 38, 75 and 43, respectively.

Conclusion: TKA is technically feasible in severely deformed neuropathic knees, with perioperative morbidity rates similar to routine TKA. The short term results of TKA in this series are encouraging, with good subjective and objective outcome scores at follow-up. TKA may be an acceptable alternative to arthrodesis in this group of patients.

I145/SOGD

Systemic Autonomic Function in Subjects with Primary Angle-closure Glaucoma

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Aim: Autonomic dysfunction is thought to be a contributory factor in primary angle closure (PAC), with autonomic neuropathy possibly involved in precipitating pupil block in anatomically predisposed eyes. This study aims to compare systemic autonomic function between subjects who have suffered a previous episode of acute angle closure (symptomatic PAC), those with asymptomatic chronic angle closure glaucoma (asymptomatic PAC), and age- and sex-matched controls.

Methods: Exclusion criteria were the use of salbutamol or ipratropium bromide, oral or topical beta-blockers, topical pilocarpine and those on systemic anticholinergic or antidepressant medications. Tests for systemic parasympathetic function included the heart-rate response to standing (ratio of 30:15), heart-rate variation during deep breathing, and the ratio of the heart rate at phases IV and II of the Valsalva manoeuvre (Valsalva ratio). For assessment of the sympathetic nervous system, blood pressure was recorded supine and then after 2 and 5 minutes of standing. A modified sweat test, the sympathetic skin response, was recorded on the palm and sole.

Results: A total of 30 subjects were examined: 8 symptomatic PACs, 8 asymptomatic PACs and 14 control subjects. The mean ages were similar and all, except 1 subject, were Chinese. There was no difference in the sex ratio between the groups. There was no significant difference ($p>0.05$) found between groups for the 30:15 ratio, heart rate variation during deep respiration and the Valsalva ratio. No significant orthostatic hypotension was detected in any subject. The occurrence of abnormal sympathetic skin response was slightly higher in the control group.

Conclusion: Our data suggests that in subjects with acute and chronic primary angle closure glaucoma, the autonomic nervous system is normal.

I146/SOGD

Allogenic Immunogenicity of Mesenchymal Stem Cell and Its Differentiated Osteogenic Lineage

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Aim: We evaluate the immunogenic reaction of allogeneic lymphocytes on mesenchymal stem cells (MSCs) and differentiated osteogenic lineage (DOL) from the same MSCs.

Methods: MSCs were purified from New Zealand white rabbits' iliac marrow. To verify MSCs, pluripotent differentiation to osteogenic lineage and chondrogenic lineage was examined. The osteogenic lineage differentiation was confirmed by positive Von Kossa staining, ALP and osteocalcin expression in ELISA, and chondrogenic lineage differentiation by positive toluidine staining and collagen-II expression in ELISA. Mononuclear cells were isolated from the rabbits' peripheral blood and spleens, then mixed with MSCs or DOL and incubated for 5 days. Proliferation of responding lymphocytes (RL) in the mixed culture was examined by CellTiter 96® non-radioactive cell proliferation assay with MTS. Cytotoxicity of stimulated RLs to MSCs and DOL was examined by CytoTox 96® non-radioactive cytotoxicity assay. MSCs and DOL from 3 different rabbit donors were cross-tested against allogeneic RLs. RLs and primary explanted osteoblasts (PEOs) from

the same donors were cross-tested against allogeneic RLs as positive controls. Autologous tests as negative controls. All experiments were performed a minimum of 3 separate times.

Results: Allogeneic RL proliferation and cytotoxicity against MSCs were significantly lower than positive controls ($p<0.01$, ANOVA) and comparable to autologous controls. Proliferation and cytotoxicity response of allogeneic RL to DOL: lower than allogeneic RL-RL ($p<0.05$) positive control, but insignificantly lower than PEO-RL ($p>0.05$) positive control and higher than autologous controls ($p>0.05$).

Conclusion: Rabbit MSCs have been observed to fail to elicit an immunogenic response from allogeneic lymphocytes. Rabbit DOL elicited little proliferation and cytotoxicity response from allogeneic lymphocytes, but needs to be investigated further in larger samples.

I147/SOGD

The Effect of Food Simulants on the Bond Strength of Orthodontic Brackets Using Glass Ionomer Cement

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Aim: We evaluate the effect of food simulants on the bond strength and failure modes of orthodontic brackets using glass ionomer cement (GIC) (Fuji Ortho LC®).

Methods: Orthodontic brackets were bonded onto 140 extracted premolars. The bonded specimens were then randomly distributed into 5 groups and conditioned in the following food-simulating liquids at 37°C: distilled water (control), 8% alcohol (aqueous food), 50% alcohol (alcoholic foods), pH4 citric acid (acidic foods) and corn oil (fatty food). The solutions were changed weekly over a period of 12 weeks. The specimens were then potted into acrylic moulds using a customised archwire-based fixture. The brackets were debonded with a shear-peel load using an Instron testing system at a crosshead speed of 0.5 mm/min. An image analysis system was used to determine the mode of failure, which was classified using the Adhesive Remnant Index (ARI). Bond strength data was analysed using 1-way ANOVA/Tukey's test and the Kruskal-Wallis test was used to determine differences in ARI data.

Results: Conditioning in corn oil significantly increased the bond strength of orthodontic brackets using GIC. No significant difference in bond strengths was observed among all other treatment groups. ARI data revealed cohesive failure for all groups.

Conclusion: Corn oil increased the bond strength of orthodontic brackets bonded with GIC. Alcohol and acidic foods do not influence the bond strength of brackets using GIC. The mode of failure was cohesive for all groups.

I148/SOGD

Transit-Time Flow Measurement is Essential to Coronary Artery Bypass Grafting

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Aim: Patent bypass grafts are fundamental to successful coronary artery bypass grafting (CABG). Intraoperative flow measurement through newly constructed grafts is a test of patency. We studied the use of transit-time flow measurement (TTFM) to determine its ability to detect technical errors in grafts; the mean flow norms for Asian patients; whether native coronary run-off can be used as a predictor of mean flow; and to compare arterial and vein grafts.

Methods: From January 2001 to September 2002, 116 patients underwent isolated primary CABG under a single surgeon. 67 underwent conventional CABG and 49 had off-pump CABG. There were 168 arterial grafts and 178 vein grafts. TTFM was carried out on all completed grafts. Graft patency was assessed using flow curves, mean flow and the Pulsatility Index (PI). The average of mean flows was calculated to determine mean flow norms. Arterial and vein grafts were compared by statistical analysis between variables of mean flow and PI. In a sample of 19 patients, the correlation between run-off

and mean flow was studied

Results: In 6 patients with 7 grafts, intraoperative graft assessment detected technical errors. These were corrected. Average mean flow was 35.66 ± 23.853 ml/min for LAD-LIMA grafts, with values ranging from 21.22 to 35.97 ml/min for the rest. There were no statistically significant differences in mean flow or PI between arterial and vein grafts. No clear relationship between mean flow and run-off could be found.

Conclusion: TTFM enables technical problems to be diagnosed accurately, allowing prompt revision of grafts. It should be mandatory in CABG to improve surgical outcomes.

I149/SOGD

Off-Pump Coronary Artery Bypass Grafting is the Preferred Choice for Patients Requiring Coronary Revascularisation

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Aim: Off-pump coronary artery bypass grafting (OPCABG) is gaining widespread acceptance as the preferred procedure for surgical myocardial revascularisation. However, no definite data exist as to whether it is better than conventional CABG. We wanted to investigate if OPCABG is indeed better than conventional CABG.

Methods: Between January 2000 and November 2002, 1020 patients underwent isolated coronary artery bypass in our institution. 167 patients underwent OPCABG. Patients were prospectively risk-stratified to Parsonnet, Euroscore and Northern New England models. Post-operative complications, ICU stay, hospital stay, types of grafts done, adoption rates amongst various surgeons were analysed.

Results: The incidence of off-pump procedures has shown a gradual increase over the last 2 years in this institution. A reduction in the numbers of post-operative complications, hospital ICU stay and mortality in the off-pump group was observed. However, most of the differences did not reach a level of statistical significance. A randomised trial is needed to prove a significant difference and benefit with the off-pump technique.

Conclusion: In conclusion, OPCABG is a viable alternative and possibly preferred to conventional CABG.

I150/SOGD

Pre-operative Eradication of Bacterial Carriage Reduces Post-operative Infections after Coronary Artery Bypass Grafting

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Aim: We investigate if the pre-operative elimination of nasal, axillary and inguinal carriage of important pathogens prior to coronary artery bypass grafting (CABG) would reduce post-operative wound infection.

Methods: 1155 consecutive patients undergoing isolated, elective CABG were included in this retrospective study. From 1 June 1995 to 30 July 1997, 312 patients (Group I) had nasal, axillary and groin swabs performed to assess bacterial load; however, bacterial colonisation was not treated. From 1 August 1997 to 30 October 2000, 843 patients (Group II) underwent pre-operative swabbing and eradication of bacterial carriage by administration of mupirocin. Eradication of bacterial carriage was confirmed by repeated cultures. Both groups underwent identical pre-operative preparation.

Results: There were 11 (0.95%) cases of sternal wound infections (SWI) between both groups of patients. In Group I, there were 6 cases (1.92%) of SWI; 3 (0.96%) patients had superficial sternal wound infections (SSWI) and 3 had deep sternal wound infections (DSWI). The incidence of SWI was significantly lower in Group II ($p < 0.05$). There were 5 (0.59%) cases of infection in this group. 2 (0.24%) were SSWI and 3 (0.35%) were DSWI.

There was a similar decrease in leg wound infections in Group II compared to Group I. There were 28 (3.3%) cases of leg wound infection versus 22 (7.1%) cases ($p < 0.01$), respectively.

Conclusion: Eradication of bacterial carriage with topical antibiotics prior to surgery reduces the incidence of postoperative wound infections among patients undergoing CABG. A large prospective, randomised, placebo-controlled study should be done to confirm this finding.

I151/SOGD

The Role of the p53 Tumour Suppressor Gene, bcl-2 and Bax-mediated Apoptosis in Gastric Cancer

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Aim: Apoptosis is a highly regulated form of programmed cell death in various tumours. The p53 tumour suppressor gene is believed to be involved in this process. Our aim was to investigate the alterations of p53 in gastric cancer and to correlate this with the expression of apoptosis-related antigens and apoptotic cell death.

Methods: Archival samples of gastric carcinomas from 232 patients were examined for overexpression of p53 by immunohistochemistry. The p53 mutations were studied by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis and sequencing. The overexpression of the bcl-2 and bax proteins was examined by immunohistochemistry. The TUNEL technique was used to determine the presence of apoptosis.

Results: There were 38.8% (using DO-7 anti-p53 antibody) and 34.1% (using 1801 anti-p53 antibody) p53 protein overexpression. Bcl-2 and bax expression was found in 30.6% and 57.3%, respectively. Apoptotic cells were found in 35%. p53 mutations were detected in 56 (24.1%) of 232 samples, in exon 5 (10.3%), exon 6 (1.3%), exon 7 (5.6%), and exon 8 (6.5%). No mutations were detected in exons 4 and 9. Polymorphism was found in codon 72 of exon 4 (CGC to CCC) in 23%.

Conclusion: The p53 gene is strongly implicated in the tumorigenesis of gastric cancer, possibly by regulating apoptosis through the expression of the apoptosis-associated genes bcl-2 and bax. Our findings have led to greater understanding of the role of p53 in gastric cancer and has important implication in prognostication and treatment using p53-mediated gene therapy.

I152/SOGD

The Role of Urodynamic Study in the Evaluation of Young Men Presenting with Lower Urinary Tract Symptoms

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Aim: We review the role of urodynamic study in the evaluation of young men who present with lower urinary tract symptoms (LUTS).

Methods: This was a retrospective study of 40 male patients presenting between September 1999 and July 2002, aged 55 years and below with no significant neurologic history, with persistent LUTS despite initial medical therapy. All patients underwent urodynamic study.

Results: The mean age of the patients was 39.4 years; 65% and 75% of them had obstructive and irritative symptoms, respectively. 47.5% of patients were found to have urodynamic abnormalities. The urodynamic findings were bladder outlet obstruction in 8 (20%) patients, detrusor overactivity in 3 (7.5%) patients, bladder outlet obstruction and detrusor overactivity in 1 (2.5%) patient, detrusor underactivity/acontractility in 4 (10%) patients and equivocal for obstruction in 3 (7.5%) patients. They were treated according to their symptoms and urodynamic findings. Patients with bladder outlet obstruction were treated with alpha-blocker therapy or transurethral surgery. Patients with detrusor overactivity were treated with anticholinergics, while those with detrusor underactivity/acontractility were treated with clean intermittent catheterisation or indwelling urinary catheter.

Conclusion: Young men presenting with LUTS may have similar symptoms but different pathologies. Urodynamic study has a definite role in the evaluation of this group of patients as it helps in the selection of definitive treatment.

I153/SOGD**A Selective Approach Increases the Clinical Benefit of Pre-operative Staging Laparoscopy and Laparoscopic Ultrasound for Gastric Cancer**Choon Hou HO¹, Melvin LOOK², Cheng Hock LOW²¹Department of General Surgery, Tan Tock Seng Hospital, Singapore, ²Department of Surgery, Tan Tock Seng Hospital, Singapore

Aim: Pre-operative laparoscopy has been found to be a highly accurate tool for staging gastric cancer. The use of laparoscopic ultrasound adds further information to this assessment. Nevertheless, the clinical impact of laparoscopic staging, based on an all-comers policy, is low as most patients do not benefit from this additional procedure. The aim of our study was to improve the clinical utility by a selective approach.

Methods: After conventional staging modalities, patients were selected for staging laparoscopy if they had a potentially curable tumour that is T3 or greater. Patients were excluded if they had a small tumour or if palliative resection was indicated. The procedure was done as day surgery whenever possible.

Results: Twenty patients with gastric adenocarcinoma satisfied our selection criteria and underwent staging laparoscopy. Laparoscopic ultrasound was used in 6 patients to obtain further information. Peritoneal metastasis was found in 10 patients. Nodal metastasis, malignant ascites and positive peritoneal lavage cytology were found in 3, 7 and 1 patients, respectively. Overall, 12 patients were found to have incurable disease and spared from unnecessary laparotomy. Two patients benefitted from palliative laparoscopic bypass done at the same sitting. There were no procedural-related complications, except for aspiration pneumonia in 1 patient.

Conclusion: Pre-operative staging laparoscopy and laparoscopic ultrasound are safe. A selective approach increases the clinical benefit of this procedure. The management of 60% of patients were altered based on this staging. Further advantage is gained if the expertise for palliative laparoscopic bypass is available.

I154/SOGD**Should Adenocarcinoma and Squamous Cell Carcinoma of the Oesophagus Be Studied Separately in Trials of Multimodality Therapy?**Khong Hee LIM¹, Wendy BROWN², Bryan BURMEISTER², Jennifer HARVEY², Ian MARTIN², David GOTLEY², Mark SMITHERS²¹Department of General Surgery, Tan Tock Seng Hospital, Singapore, ²Upper Gastrointestinal Surgical Unit, Department of Surgery, University of Queensland, Princess Alexandra Hospital, Australia

Aim: Multimodality therapy has been used to treat oesophageal cancer. We compared the treatment outcome between adenocarcinoma (AC) and squamous cell carcinoma (SCC) in terms of survival and recurrence.

Methods: A prospective database of patients with oesophageal cancer was collected from 1988-2001. 284 patients with AC and 191 patients with SCC were treated with curative intent. The options included resection alone, neoadjuvant chemotherapy with or without radiotherapy (cisplatin 80 mg/m², fluorouracil 800 mg/m² and 35 Gy) followed by resection, and definitive chemoradiotherapy (2 cycles of cisplatin 80mg/m² and fluorouracil 800 mg/m² and 60 Gy).

Results: The 2-year survival rates of patients with AC and SCC treated by resection were 43.6% and 35.9%, respectively (P = 0.352). Patients with SCC had a better 2-year survival rate (69.2%) than patients with AC (35.6%) receiving neoadjuvant therapy (P = 0.009). Patients with SCC treated by definitive CRT also had a better 2-year survival rate (42.4%) than those with AC (26.2%) (P = 0.024). Patients with AC treated with neoadjuvant therapy or definitive CRT had a higher recurrence rate than those with SCC. Distant recurrence was the major failure in both histologic groups.

Conclusion: The results of resection of SCC and AC of the oesophagus were comparable. However, when CRT is included in the treatment, either as pre-operative or definitive therapy, the outcome was significantly different. Although there may have been selection bias in the various treatment groups, the results suggest that future trials that involve CRT should consider the different histologic types separately.

I155/SOGD**DVT in an Asian Population**Ker Kan TAN¹, Woon Puay KOH², Alexandre K H CHAO¹Department of General Surgery, Tan Tock Seng Hospital, Singapore, ²Department of Community, Occupational and Family Medicine, National University Hospital, National University Singapore, Singapore

Aim: Deep vein thrombosis (DVT) is perceived to occur less frequently among Asians than Caucasians, although the reason for this has not been fully understood. This study examined the association of classical DVT risk factors and presentation among Asian patients with DVT.

Methods: We retrospectively reviewed 862 symptomatic patients referred to the vascular diagnostic laboratory for suspected DVT over a 30-month period. 271 patients diagnosed with DVT on ultrasound were matched for age, gender and ethnic group to negative controls to study the significance of risk factors and presenting symptoms or signs.

Results: Of the population screened, 74.7% were Chinese, 10.5% were Malay, 12.3% were Indian and other races constituted 2.5%. 277 (32.1%) patients tested positive for DVT on duplex ultrasound. In a multivariate analysis, independent risk factors for DVT included immobility (OR 2.61; 95% CI 1.63-4.15), malignancy (OR, 1.77; 95% CI, 1.01-3.10) and history of thrombophilia (OR, 9.95; 95% CI 1.26-78.87). The significant forms of DVT presentation were limb swelling (OR, 2.53; 95% CI, 1.41- 4.54) and a history of pulmonary embolism (OR, 11.45; 95% CI, 3.23-40.59). Fever >37.5°C was a negative predictive factor (OR, 0.42; 95% CI, 0.23-0.76).

Conclusion: The findings of this study show that the common risk factors of DVT, such as surgery and fractures, do not affect Asians as much as Caucasians. Thrombophilia and underlying malignancy are prominent risk factors among Asians, and a case of DVT in an Asian with no apparent cause should prompt the clinician to look for these predisposing factors.

I156/SOGD**Study of 201 Cases of Bladder Calculi: Combined Endoscopic Surgery for Bladder Calculi and Bladder Outlet Obstruction Will Not Cause Excessive Morbidity**

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Aim: Bladder calculi are frequently associated with bladder outlet obstruction from benign prostatic hyperplasia. Hence, patients often require both endoscopic lithotripsy and transurethral resection of the prostate (TURP). There are concerns that the morbidity of combining endoscopic lithotripsy and TURP is excessive. We aim to determine whether combining the 2 procedures leads to excessive morbidity.

Methods: This was a retrospective study on 201 consecutive operations for bladder calculi, comprising 97 cases of endoscopic lithotripsy and 104 cases of lithotripsy and TURP. 73 patients who underwent TURP during the same time period were selected as a control group.

Results: The addition of TURP significantly increased the complication rate (21.2% for the combined procedure compared to 8.2% for lithotripsy alone). However, the increased complication rate of lithotripsy and TURP was not significantly different from that of the control group who underwent TURP alone (21.2% vs 20.5%). An increased amount of prostatic resection was found to be a predisposing factor for complications from lithotripsy and TURP.

Conclusion: These findings support the practice of combined lithotripsy for bladder calculi and TURP at the same sitting, with the caveat that operating on patients with large prostates may lead to increased risk of complications.

I157/SOGD**An Expert System Using Artificial Intelligence Case-based Reasoning for the Prediction of Outcome in Acute Upper Gastrointestinal Bleeding**Melvin LOOK¹, Yah Yuen TAN¹, Appasamy VIJAYAN¹, Geok Hoon NG², San Nee FONG², Seng Joo THIO², Lean Suan ONG²¹Department of General Surgery, Tan Tock Seng Hospital, Singapore, ²Institute of Systems Science, National University of Singapore, Singapore

Aim: The accurate early prediction of outcome in acute upper gastrointestinal

bleeding is important for appropriate, cost-effective treatment. Conventional risk scoring by statistical models is inflexible, inaccurate and lack clinical intuition. Our aim was to evaluate a decision support tool that reasons and solves complex problems using Artificial Intelligence case-based reasoning. **Methods:** Data from patients' biodata, past history, co-morbidity, presentation, investigation, endoscopy, treatment and daily progress formed 86 fields representing a case. Useful fields were weighted and used to compute similarity scores by nearest neighbour matching. The predicted outcome is expressed as a probability of rebleeding. Actual patient data from 512 historical cases formed the database. Another 95 patients were used as test cases.

Results: Prediction was undertaken early in the course of admission (on the day of endoscopy). Out of 68 cases predicted to have low risk of rebleeding, there were 2 actual cases of minor rebleeding. Out of 13 predicted intermediate-risk cases, there was 1 actual case of minor rebleeding, 1 actual case of readmission from rebleeding and 1 actual mortality. Out of 14 predicted high-risk cases, there was 1 actual case of minor rebleeding, 1 actual case of readmission from rebleeding and 1 actual mortality. Validating the prediction by testing all 607 cases in the database against itself showed a similar degree of accuracy.

Conclusion: Outcome predictions were found to be useful in clinical decision-making. This accuracy will improve when the system "learns" as the database increases. This is a landmark study showing the novel and beneficial application of Artificial Intelligence in a medical domain.

I158/SOGD

Pure Laparoscopic Urologic Surgery: Review of Indications, Approaches and Complications

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Aim: We review our initial experience in pure laparoscopic urologic surgery in Tan Tock Seng Hospital.

Methods: Forty-eight consecutive cases of pure laparoscopic urologic surgery done from August 2001 to March 2003 were reviewed with respect to indications for surgery, age and sex of subjects, surgical approaches, length of procedure, length of hospital stay, complications, reasons for conversion to open surgery, final pathology and clinical outcome. One hand-assist laparoscopic nephroureterectomy was excluded from this review.

Results: There were 25 cancers, 6 non-malignant tumours and 17 benign conditions. Of all the cancers, 17 were radical nephrectomies, 4 were nephroureterectomies, 1 partial nephrectomy and 3 radical prostatectomies. Majority (36) of the cases were done via the transperitoneal approach. Six were retroperitoneal and 6 were pre-peritoneal in approach. While the majority were ablative in nature, there were reconstructive cases: like 3 prostatectomies, 1 pyeloplasty and 1 ureterolithotomy. There were 6 cases of groin hernia repair using the non-stapled anatomical polyester slit mesh. In ablative cancer surgery, specimens were usually extracted through a Pfannestiel incision. Reconstructive cases were done using intracorporeal suturing. Operative time ranged from 1 (diagnostic) to 9 (radical prostatectomy) hours. The average length of stay varied with pathology. Four cases (8%) were converted to open surgery. No major intraoperative complications and mortality were encountered in our early series.

Conclusion: Familiarity with various surgical approaches allows a wide spectrum of urologic pathology to be managed by pure laparoscopy. Our early experience indicates that the learning curve of pure laparoscopy can be mounted with acceptable complications and conversion rate.

I159/SOGD

Initial Experience of Adjuvant Chemoirradiation after D2 Gastrectomy for Gastric Cancer

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Aim: We determine the 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 of 425mg of fluorouracil per square metre of body surface area per day plus 20 mg of leucovorin per square metre per day, for 5 days, followed by 4500 cGy of radiation at 180 cGy per day, given 5 days per week for 5 weeks, with modified doses of fluorouracil and leucovorin on the first 4 and last 3 days of radiotherapy.

Results: The total number of patients was 37 with a median age of 62 years. 29 patients had undergone subtotal gastrectomy. 32% of patients developed mucositis and 78% had gastrointestinal side effects. 38% of patients experienced weight loss. 27% of patients were admitted during the chemoirradiation regime for neutropenic sepsis, gastrointestinal disturbances and deep vein thrombosis for 1 patient. A total of 29 patients completed the chemoirradiation regime.

Conclusion: 78% of patients completed the chemoirradiation regime, reflecting good tolerability even after D2 gastrectomy. None of the patients suffered major toxic side effects; most of the side effects were related to chemotherapy, of which gastrointestinal disturbances were the most common. Follow-up with longer duration is required to study the impact of adjuvant therapy on disease progression and survival.

I160/SOGD

The Virtual Reality Colonoscopy Simulator as a Skills Assessment Device

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Aim: Computer-based Virtual Reality (VR) medical simulation has emerged as a useful tool in teaching skills that require dexterity and eye-hand coordination. Numerous summary performance measures can also be recorded. This is useful, but the validity of VR simulators as a measure of what it purports to measure remains unproven. Our aim was to study the validity of the VR colonoscopy simulator as a skills assessment device by using its construct validity as a surrogate measure, that is, whether this test can distinguish those with the desired skills from those without.

Methods: Three groups of subjects (37 novices, 14 trainees and 9 experts) underwent 2 standardised tests and were scored on 5 objective parameters and a composite score that we devised.

Results: Performance scores increased with expertise for the composite score (P < 0.001), time score (P < 0.001) and depth of insertion score (P = 0.02), but not for mucosa visualisation score, red-out score or pain score (Spearman's rank correlation test). Amongst the novices, there was no difference in their performance on the test in relation to gender or time spent on other pursuits requiring psychomotor skills, such as arcade games, sports, playing musical instruments and handicrafts (Mann-Whitney U test).

Conclusion: The VR colonoscopy simulator is a good skills assessment device. The useful parameters to assess are the time score, depth of insertion score and composite score. It is also unlikely that the innate ability of a prospective trainee can be predicted by his aptitude for other related activities.

I161/SOGD

Renal Cell Carcinoma: A 10-year Review in a General Hospital

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Aim: A 10-year retrospective analysis of renal cancers diagnosed at Tan Tock Seng Hospital between 1993 and 2002 was undertaken.

Methods: A review of patients notes who had been diagnosed with renal cell carcinoma between January 1993 and December 2002 was performed. In particular, pathological staging and grade were analysed.

Results: 159 patients were reviewed. 22.6% were Stage I, 22% Stage II, 24.5% Stage III and 30.8% Stage IV at presentation. Mean age at diagnosis was 64.4 years and there was no downward trend in the mean age at diagnosis during the study period. 96 underwent either radical or partial nephrectomy, of which 17 were performed laparoscopically in the last 18 months. The most common renal cell carcinoma histologic subtypes were clear cell carcinoma (83.3%) and papillary renal cell carcinoma (6.3%). Fuhrman histological grade in 88 patients was compared with their respective T stage and showed

that poorly differentiated tumours are associated with higher T stage, whereas better differentiated tumours are associated with lower T stage, with strong statistical significance.

Conclusion: The clinical and pathological profile of our study population is comparable to other published series. The Fuhrman histological grade in renal cell carcinoma is significantly associated with the T stage.

I162/SOGD

Endovascular Stent Grafting of the Thoracic Aorta: The National Healthcare Group Experience

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Aim: We review the results of endovascular stent grafting procedures of the thoracic aorta in the National Healthcare Group.

Methods: A retrospective chart review of 17 patients over a 36-month period was performed.

Results: The mean age of our patients is 62.7 years (range 29 to 78 years). There were 13 male and 4 female patients. Six patients had chronic Type B dissections with expanding aneurysms, 1 had an acute Type B dissection with leak into the left hemithorax and 5 patients had leaking saccular aneurysms. One patient had a transection of the thoracic aorta after a fall from height and another had acute dissection after a road traffic accident. Twelve cases (70.1%) were done in the Angiography Suite. The mean operating time was 187 minutes (range 120 to 300). The average blood transfusion requirement was 1 pint (range 0 to 5). Three patients required intensive care. The median post-operative stay was 10 days (range 4 to 22 days). The 30-day mortality was 5.9% (1 patient with leaking thoracic aneurysm who developed CVA post-procedure). There were no paraplegia and no other major adverse cardiac events. The patient with the haemothorax from the leaking dissection had to be re-admitted for management of a persistent pleural collection. Follow-up ranged from 1-28 months. One patient required stenting of a late type I endoleak from migration (6.7% reintervention rate). Two patients succumbed from unrelated causes.

Conclusion: Endovascular stenting can be performed safely with low morbidity in terms of blood transfusion requirements, reduced ICU stay and minimal complications even in an emergency setting.

I163/SOGD

Groin Pain and Sports Hernia in Professional Footballers in Singapore

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Aim: Groin pain is a common sports-related problem, especially among professional footballers. Diagnosis is difficult as it can be due to various causes. We aim to define the extent of the problem among professional footballers in Singapore, the impact on their playing careers and related aetiology.

Methods: A prevalence study of 26 male professional footballers from a single premier division club in Singapore. The biodata, playing history, injury history and diagnosis were obtained by clinical review and physical examination.

Results: The median age was 22 years (range 19 to 31). The mean number of years spent in amateur and professional soccer were 5.3 and 5.2 years, respectively. 14 players (53.9%) had a history of groin pain. Of these, 6 (42.9%) had an active problem and 8 (57.1%) had recurrent groin pain. Playing performance was affected in most players, including enforced rest in 6 (42.9%) players. The average and maximum time out was 5 (1 to 14) weeks and 5.5 (1 to 20) weeks, respectively. The aetiology of groin pain was found to be sports hernia in 7 players (50%). Other causes were adductor strain and tears, acute groin strain and rectus femoris tear in 6 (42.9%), 2 (14.3%) and 1 (7.1%) player, respectively. 2 players had more than 1 problem.

Conclusion: Groin pain is common among professional footballers and has

a significant impact on performance. Sports hernia is a common cause. Awareness and accurate diagnosis are important so that proper treatment, including surgery, is given.

I164/SOGD

Single Finger Distal Amputation: Replant or Terminalise? Comparison of Costs and Functional Outcome

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Aim: Replantation of distal amputations provides the best functional and cosmetic reconstruction of the fingertip, but is technically challenging, resource- and cost-intensive. Terminalisation of the distal phalanx of a single digit is simpler and cheaper, with little loss of function, except in thumb amputations. This study compares the cost-effectiveness of single digit distal replantation and terminalisation.

Methods: All patients with single digit distal amputations, no other injuries or contraindications to replantation and were treated by replantation or terminalisation between 2001 and 2003 were included. Direct costs of treatment and rehabilitation and indirect costs from loss of productivity and compensation were compared. Patients' satisfaction with cosmesis and function was assessed. Function was assessed clinically, and with a Purdue pegboard time test to compare fine manipulation of the injured and uninjured hands. Patients were assessed when they were ready for work, a minimum of 3 months after surgery.

Results: 36 fingertips were replanted, 78% of which remained viable. 31 were terminalised. The direct costs of an uncomplicated replantation were 4.94 times that of an uncomplicated terminalisation. After including a prosthesis for terminalised fingers, this ratio dropped to 2.47. Duration of medical leave and light duties was 2.48 times greater for uncomplicated replantation. Compensation for disability was not significantly different. Replantation provides better fine manipulation and precision pinch, less hypersensitivity and cold intolerance, and more satisfying function and cosmesis.

Conclusion: Single digit distal replantation is costlier than terminalisation but is justified, providing superior function and cosmesis, with higher patient satisfaction.

I165/SOGD

Biological Spare Part Surgery

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Aim: Reconstruction after complex hand injuries remains a challenge to the reconstructive surgeon. This is because of the limitation of ideal donor tissues and donor morbidity. We need to balance satisfactory outcome with minimal donor morbidity. The problem arises when more than 1 digit needs to be reconstructed. We utilised biological tissues of compromised function from the same injured hand for reconstruction, which is known as "biological spare part surgery".

Methods: We present 2 cases in which we split a digital stump from the same hand into parts for multiple digit reconstructions. Our first patient sustained traumatic amputations of his left index, middle and ring fingers. We transferred the ring finger to the index and middle fingers by splitting it longitudinally into 2 parts, and anastomosing it as a syndactylised unit. Six months later, release of syndactyly was performed. Our second patient sustained traumatic amputations of bilateral index and middle fingers. Reconstruction was performed by segmentally dividing the right index finger, transferring the distal stump to the left index finger and the proximal part to the right middle finger.

Results: In both cases, we were successful in improving function as well as cosmesis to the hands. As the tissues used were taken from the same hand, donor morbidity was negligible.

Conclusion: This concept of "spare part" surgery, using 1 donor digit for 2 digital reconstructions, may resolve the problem of availability of donor

tissues while keeping donor morbidity to a minimum. These 2 cases represent a new milestone in reconstructive microsurgery.

I166/SOGD

Quantification of *Actinobacillus Actinomycetemcomitans* in Periodontal Health and Diseased

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Aim: *Actinobacillus actinomycetemcomitans* is associated with aggressive forms of periodontal disease. A great utility to study the aetiological and pathological aspect of this bacterial species in periodontal disease is the ability to make quantitative assessment of the bacteria at various disease stages. In comparison to conventional culture method, real-time PCR offers a faster, more sensitive and efficient alternative to quantitation of bacteria. We aim to study the feasibility of quantitating *A. actinomycetemcomitans* from subgingival plaque using real-time PCR and to compare the quantities of *A. actinomycetemcomitans* obtained from periodontally healthy and diseased subjects.

Methods: Subgingival plaque samples were obtained from 20 chronic periodontitis and 15 periodontally healthy subjects. The assay uses SYBR Green to continuously monitor product formation. Optimum parameters of primer sequences, concentrations of primers and/or templates and PCR conditions were experimentally determined. A standard curve was generated using DNA template corresponding to known numbers of bacteria. Plaque samples were assayed and compared with the standard curve to determine the quantity of bacteria cells present.

Results: A linear quantitative detection range was obtained with a detection limit of 10 cells. Eleven healthy subjects (73%) and 17 diseased patients (85%) tested positive for *A. actinomycetemcomitans*. The amount of *A. actinomycetemcomitans* in plaque samples ranged from 102 to 106 in both periodontally healthy and diseased, with modes of 103.

Conclusion: We showed that quantitation of *A. actinomycetemcomitans* from plaque samples using real-time PCR was possible. The amount of bacteria did not vary significantly between periodontally healthy and diseased subjects in ethnic Chinese. This study was supported by grants R-182-000-044-213 and R-222-000-012-112 from the National Medical Research Council and the National University of Singapore, respectively.

I167/SOGD

Management of Ruptured and Unruptured Symptomatic Posterior Inferior Cerebellar Artery Aneurysms

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Aim: Distal posterior inferior cerebellar artery (PICA) aneurysms are very rare. We analysed our series of eight consecutive patients to evaluate their management and outcome, with emphasis on parent artery sacrifice without revascularisation.

Methods: Eight consecutive ruptured and unruptured distal PICA aneurysm patients from January 1998 to December 2002 were reviewed retrospectively.

Results: The mean age was 49.1 ± 7.4 years. Two were unruptured aneurysms and 6 presented with acute subarachnoid haemorrhage. Four presented with WFNS Grade 5 with the other 4 grade 1 and 2. All the patients with ruptured aneurysms were subjected to cerebral angiograms on the day of admission. All surgeries were performed immediately after angiogram through the far lateral approach. Six were fusiform aneurysms while 2 were saccular aneurysms. The saccular aneurysms were clipped, 3 fusiform aneurysms were trapped and 2 proximal clippings done. Three patients with proximal aneurysms had their parent artery sacrificed. Six out of seven operated patients needed CSF shunting for hydrocephalus. All check angiogram during follow-up did not show recurrence of aneurysm. None developed the PICA syndrome or showed infarction on brain scans. All good grade patients recovered without neurological deficits. The only major morbidity was from shunt infection.

Conclusion: Our experience showed that early surgery for ruptured PICA aneurysm carries good prognosis and low morbidity. The need for revascularisation procedures in proximally located PICA aneurysms is contrary to conventional wisdom, but only a larger series would likely demonstrate the factors in predicting whether the parent vessel could indeed be sacrificed safely without neurological sequelae.

I168/SOGD

Expression of Poly-ADP Ribose Polymerase and its Link with Apoptosis-related Cell Death in Human Traumatic Brain Injury

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Aim: Inhibition of PARP has been shown to be neuroprotective in animal models of cerebral ischaemia. However, PARP activation has yet to be addressed in humans. We investigated the cellular pattern of PARP expression during human head injury and its relation to apoptosis-related cell death, and attempted to correlate it with clinical parameters.

Methods: Patients with mass lesions and refractory intracranial hypertension who underwent emergency craniotomy and evacuation were recruited into the study. Paraformaldehyde-fixed specimens of peri-contusional tissue obtained from 16 head-injured patients were subjected to PARP immunohistochemistry. The numbers of positively-stained cells (x20 magnification field) were counted manually. The clinical parameters analysed were admission GCS, time interval between injury and surgery, initial ICP and ICP values at 6, 12 and 24 hours after surgery. Statistical analysis was carried out using SPSS.

Results: In each of the 8 specimens that were found suitable for analysis, activated Caspase-3 immunoreactivity was present in all samples. PARP immunoreactivity was present exclusively in the nucleus of some neurons, whilst other neurons showed both nuclear and cytoplasmic localisation. Nuclear staining of PARP significantly correlated with a short time interval to surgery (<4 hours), but not with admission GCS, initial ICP or subsequent ICP values.

Conclusion: Cytoplasmic localisation of PARP may reflect PARP cleavage and, thus, ongoing apoptosis. As cytoplasmic PARP expression predominates at early time intervals (4 hours or less), this would suggest that apoptotic mechanisms of cell death are active in an early time frame following head injury.

I169/SOGD

Management of Aneurysmal Subarachnoid Haemorrhage in Elderly Patients

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Aim: The management of subarachnoid haemorrhage (SAH) from ruptured cerebral aneurysms consumes considerable acute management and rehabilitation resources. We undertook a retrospective study of the elderly patients to identify factors contributing to mortality and poor outcome.

Methods: A retrospective review of elderly aneurysmal SAH patients (65 years old) presented to the National Neuroscience Institute from December 1998 to January 2001 was done. The outcome was categorised with the Glasgow Outcome Scale (GOS) at 6 months post-ictus.

Results: There were 48 patients with a mean age of 74.15 ± 5.99 years. The male to female ratio was 1:15. One patient without the GCS on admission was excluded from the analysis. The patients with WFNS grade 1, 2, 4 and 5 were 40.4%, 10.6%, 34% and 14.9%, respectively. Forty-five percent presented with Fisher grades 3 and 4. Hydrocephalus was present in 64.6% of patients on admission and 45.8% had hyponatraemia. The mortality at 6 months was 17%. 38.3% of patients had poor outcome (GOS 2 and 3) and 44.7% had GOS 4 and 5 at 6 months. Fisher grade on admission was the only significant factor affecting the GOS at 6 months ($p = 0.002$).

Conclusion: The majority of the elderly patients with aneurysmal SAH were either dead or had poor outcome at 6 months post-ictus. The only predictor of outcome was the Fisher grade on admission. A larger series is needed to enable an outcome prediction model to be formulated to better utilise our limited resources.

I170/SOGD

Cervical Corpectomy for Cervical Spondylotic myelopathy and Ossification of the Posterior Longitudinal Ligament: Results of the First 100 Cases

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Aim: Cervical spondylotic myelopathy (CSM) with ossification of the posterior longitudinal ligament (OPLL) is a common and challenging cause of myelopathy in Oriental populations. The treatment approaches are varied and include anterior or posterior decompressive surgeries. We study the results of the first 100 cases treated at our institute by anterior cervical corpectomy and fusion with iliac crest graft (ACCF).

Methods: The first 100 patients were reviewed. The clinical notes, operative recordings, post-surgical evaluations and imaging studies were all carefully analysed. The results were compared to those achieved by other techniques reported in the literature.

Results: 97 patients improved their neurological function after surgery. 1 patient did not improve and 2 died from unrelated medical problems. The complication rate was minimal and decreased as familiarity was gained with the procedure. Compared to other reported techniques, this is the most successful technique for the treatment of CSM and OPLL.

Conclusion: ACCF is the treatment of choice for CSM and OPLL.

I171/SOGD

Paediatric Tracheostomy: The KK Women's and Children's Hospital ExperienceDennis Yu Kim CHUA¹, Henry Kun Kiang TAN²¹Medical student, National University of Singapore, Singapore, ²Department of Otolaryngology, KK Women's and Children's Hospital, Singapore

Aim: We analyse paediatric tracheostomies in KK Women's and Children's Hospital from 1997 to 2003.

Methods: A retrospective review of all case notes for infants and children who underwent tracheostomy in Kandang Kerbau Women's and Children's Hospital from 1997 to 2003 was performed.

Results: There were a total of 23 patients; their age ranged from 16 days to 14 years. Paediatric tracheostomies were mainly performed for congenital disorders resulting in upper airway obstruction. Fifteen of 23 tracheostomies were done within the first year of life. Five of the 23 patients underwent semi-urgent tracheostomies, while the rest were elective cases. Thirty-five percent of the patients had early complications whilst 70% had late post-operative complications. Chest infection was the commonest early and late post-operative complications. There were 9 attempts at decanulation and 8 were successful. Three (13%) patients who underwent tracheostomy died from causes probably not related to tracheostomy.

Conclusion: Paediatric tracheostomies were mainly done for congenital lesions. Most tracheostomies were done within the first year of life. The complication rate is high and 3 of our patients eventually died. The deaths of these patients, however, could be due to multiple co-morbidities.

I172/SOGD

Can Low-dose Oral Solution Misoprostol be Used as a Uterotonic Agent to Prevent Postpartum Haemorrhage?Yap Seng CHONG¹, Selina CHUA¹, Liang SHEN², Sabaratnam ARULKUMARAN³¹Department of Obstetrics and Gynaecology, National University of Singapore, Singapore, ²Clinical Trials and Epidemiology Research Unit, Singapore, ³Department of Obstetrics and Gynaecology, St George's Hospital Medical School, London, United Kingdom

Aim: In an earlier study, we found that misoprostol 400 mg, given as an oral solution, produced the fastest and greatest uterotonic effect compared to other routes of administration, but resulted in high rates of side effects. We hypothesised that a lower dose of oral solution misoprostol would give a better side effect profile without significant loss of uterotonic effect.

Methods: 30 women were given either misoprostol 200 or 400 mg as an oral solution, or intramuscular syntometrine 1mL after spontaneous vaginal delivery. Pre- and post-treatment uterine activities were measured with

intrauterine pressure catheters.

Results: Uterine activity produced by oral solution misoprostol 200 mg was not significantly different from that of oral solution misoprostol 400 mg ($P = 0.758$) or intramuscular syntometrine 1 mL ($P = 0.623$), with the maximum probable differences between treatment groups not statistically or clinically significant. Onset of action was also not significantly different among the 3 groups. Shivering and pyrexia occurred less frequently (20% versus 50%, $P = 0.35$; and 10% versus 90%, $P = 0.005$, respectively) and the maximum body temperatures recorded were significantly lower ($P < 0.001$), with 200 mg compared to 400 mg oral solution misoprostol.

Conclusion: The differences between the uterotonic effect produced by oral solution misoprostol 200 µg and 400 µg, and between oral solution misoprostol 200 µg and intramuscular syntometrine 1 mL, were small while the difference in side effects produced was significant. We suggest that a strategy to consider for the safe routine use of misoprostol to prevent postpartum haemorrhage would be to give low doses of misoprostol by oral solution, rather than higher doses by other routes.

I173/SOGD

Does the Route of Administration of Misoprostol Make a Difference for the Routine Prevention of Postpartum Haemorrhage? The Uterotonic Effect and Side Effects of Misoprostol Given by Different Routes after Vaginal DeliveryYap Seng CHONG¹, Selina CHUA¹, Liang SHEN², Sabaratnam ARULKUMARAN³¹Department of Obstetrics and Gynaecology, National University of Singapore, Singapore, ²Clinical Trials and Epidemiology Research Unit, Singapore, ³Department of Obstetrics and Gynaecology, St George's Hospital Medical School, London, United Kingdom

Aim: Despite the findings of recent studies that parenteral oxytocics are preferable to oral tablet misoprostol for the management of third-stage labour, interest in misoprostol remains because of its ease of use and storage and its low cost. There has, however, been little agreement on the optimum dose and route of administration of misoprostol for this purpose. We studied misoprostol 400 mg administered by different routes after vaginal delivery in order to identify the ideal route for its administration in terms of onset of action, uterotonic effect and side effects.

Methods: 50 women were given misoprostol 400 mg, either by oral solution, oral tablet, rectal or vaginal route, or intramuscular syntometrine 1 mL after vaginal delivery. Pre- and post-treatment uterine activities were measured with intrauterine pressure catheters.

Results: Uterine activity produced by oral solution misoprostol 400mg was significantly higher than that of oral tablet, rectal and vaginal misoprostol ($P = 0.045$, $P = 0.004$, $P = 0.002$, respectively). Onset of action after oral solution misoprostol was faster than by the oral tablet ($P = 0.01$), rectal ($P < 0.0005$) and vaginal ($P < 0.0005$) routes. Shivering and pyrexia were most common with oral solution misoprostol (50% and 90%, respectively). The maximum body temperature recorded was also significantly higher ($P = 0.001$) with oral solution misoprostol.

Conclusion: Different routes of administering misoprostol greatly influence the effects achieved. Oral solution misoprostol produces the fastest and strongest uterotonic effect but, unfortunately, with the most side effects. This study highlights the need to re-examine carefully the route and dose of misoprostol used for the purpose of preventing postpartum haemorrhage.

I174/SOGD

Dengue in Pregnancy: A Singaporean Perspective

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Aim: Dengue remains a serious public health problem in Singapore with frequent outbreaks among adults. It is associated with adverse fetal outcomes and maternal morbidity. This has not previously been well described in Singapore. This study evaluates the consequences of such an infection during pregnancy.

Methods: A retrospective review of patients with dengue fever managed in our hospital in the last 4 years was performed. Diagnosis was confirmed using

the ELISA test (Focus Technologies, Cypress CA). Their own obstetricians monitored the patients, with ID physician input in 3 cases.

Results: Six women with dengue were identified in this period. They were aged 31 to 36 years and developed fever between 7 to 33 weeks of gestation, with 2 patients in each trimester. All patients developed fever; 3 (50%) reported abdominal pain or discomfort, 2 (33%) reporting a rash and 1 reported headache and photophobia. They were hospitalised for an average of 3.8 ± 2.04 days. On repeated blood tests, they had a platelet nadir of 171.6 ± 38.53 . None of them required platelet transfusion. There was 1 fetal death in-utero and 5 normal vaginal deliveries with healthy infants. There were no cases of premature birth or abruptio placentae. There were no signs of vertical transmission.

Conclusion: While pregnant women with dengue are at risk of adverse fetal outcomes, careful monitoring can be associated with favourable results.

I175/SOGD

Prospective Clinical Audit of Morbidity and Mortality in Gynaecological Surgery and Obstetric Deliveries Over a 4-month Period in a University Teaching Hospital

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Aim: Prospective clinical audit of morbidity and mortality in gynaecological surgery and obstetric deliveries over a 4-month period in a university teaching hospital.

Methods: A prospective study in complications, morbidity and mortality of 410 consecutive patients who underwent gynaecological surgery or delivered a baby between 2 February 2003 and 30 May 2003 was performed. Procedures were divided into gynaecology and obstetrics. The multitude of gynaecological procedures were subdivided into minor, laparoscopic and major surgeries. Obstetric procedures were divided between vaginal and caesarean deliveries. The complications were analysed.

Results: In 410 procedures, the overall complication rate was 10.97% (44/410). Gynaecological surgery complications: overall complication rate was 15.49%. Complication rate of laparoscopy was 5.88% (1 injury to bladder), minor operation was 5.26% (1 haemorrhage, 1 atrial fibrillation) and major operation was 21.83%, with 2 deaths following complicated debulking cancer surgery. Most common was paralytic ileus (5.74%) followed by small bowel injury (3.44%). Average stay of a laparoscopic operation was 2.18 days. Obstetric complications: overall complication rate was 7.54%. Most common was postpartum haemorrhage (3.77%). Complication rate of caesarean section was 11.94% and vaginal delivery was 6.06%. Average length of admission for vaginal delivery was 2.51 days and for caesarean section, 3.65 days.

Conclusion: Complications in this institution are less common than international standards (10.97% vs. 11.6% to 14.8%). The caesarean section complication rate is higher than NVD and the length of stay longer. The study will ensure an optimal standard in procedures and allow the hospital to fully utilise resources by understanding the length of stay in various operations and the amount of OT and delivery suite usage.

I176/SOGD

Epidemiology of Charles Bonnet Syndrome in an Asian Tertiary Ophthalmic Centre

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Aim: To describe the epidemiology of Charles Bonnet syndrome (CBS) among patients in an Asian tertiary ophthalmic centre and to describe the characteristics of the hallucinations experienced.

Methods: 1000 randomly selected patients aged 50 years and above were surveyed at The Eye Institute at Tan Tock Seng Hospital. Patients who reported formed visual hallucinations were evaluated in greater detail and were asked to describe the characteristics of their hallucinations and their

reactions to it.

Results: The mean age of the patients was 67.9 years (range, 50 to 104 years). There were 457 males and 543 females. The most common ocular pathology in these patients were cataract (59.1%), diabetic retinopathy (14.3%), glaucoma (9.2%) and age-related macular degeneration (4.2%). Their visual acuity (VA) ranged from 6/6 to no light perception. Two men and 2 women described formed visual hallucinations characteristic of CBS. Their mean age was 76.3 years (range, 65 to 90 years). Two of them had cataracts, 1 had glaucoma and 1 had both cataracts and glaucoma. The VA in the patients' worse-seeing eye was 6/12, 6/18, 6/30 and hand movements. The hallucinations experienced varied and included people, animals and inanimate objects. Three of the 4 patients had discussed their symptoms with family members and had attributed the hallucinations to poor eyesight.

Conclusion: This is the first report on the epidemiology of CBS in Asian patients. The prevalence of CBS in Asian ophthalmic patients is slightly lower than in previous reports in Caucasian patients.

I177/SOGD

Visual Experience During Cataract Surgery: A Survey on the Knowledge of Singapore Optometry Students

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Aim: Recent studies have shown that many patients undergoing cataract surgery using regional or topical anaesthesia experience a variety of visual sensations with their operated eye intraoperatively, with up to 15.4% of patients finding their visual experience frightening. Since optometrists are an important source of information for patients considering cataract surgery, this study aims to determine the knowledge of Singapore optometry students on this subject.

Methods: A survey using a standardised, self-administered questionnaire on possible visual sensations experienced by patients during cataract surgery was conducted on all optometry students in Singapore.

Results: The response rate amongst the 125 students was 87.8%. The mean age of the respondents was 19.6 years (range 18 to 24 years). 37% and 38.9% believed that patients may experience no light perception under topical and regional anaesthesia, respectively, while 68.5% and 74.1%, respectively, felt that patients were likely to have light perception. More than half believed that patients might experience other visual sensations intraoperatively. 70.4% and 67.6% of students believed that patients undergoing cataract surgery under regional and topical anaesthesia, respectively, may be frightened by their visual experience, while 88% and 86.1% felt that preoperative counselling might help to alleviate the fear.

Conclusion: The majority of Singapore optometry students were aware that patients might experience various visual sensations during cataract surgery. In addition, a high percentage felt that patients may experience fear from their visual experience and would benefit from preoperative counselling.

I178/SOGD

Epidemiology of Pterygium Among Residents on a Tropical Island in Indonesia

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Aim: To describe the epidemiology of pterygium among residents of a tropical island in Indonesia and to examine the roles of age and gender as determinants of bilaterality and severity of the disease.

Methods: An eye examination was performed as part of a voluntary health screening programme for residents of Pulau Jaloh, an island in the Riau

Archipelago, Indonesia.

Results: Of an estimated 550 inhabitants, 477 (86.7%) responded to the screening and were examined. Their mean age was 22.8 years (range, 0 to 90 years, SD 18.1). The overall prevalence rate of pterygium was 17%. 71.6% of subjects with pterygium had bilateral disease, most of them in the third decade of life. 22.7% of males and 12.4% of females had the disorder, the gender difference being statistically significant. Adjusted for age, the risk of the disease was 3.1-fold higher among the males. Subjects with pterygium were significantly older; their mean age was 42.9 years compared to 16.7 years among those without disease. The prevalence rates in males increased very rapidly with age from age 20 years to reach a plateau of 63.6% at age 35 and remained stable thereafter. In the females, the rates also increased with age, albeit at a slower rate, from age 20 years to reach a plateau of 46.7% at age 55 and remained stable thereafter.

Conclusion: There is a high prevalence rate of pterygium among the study subjects, with the rates increasing rapidly with age.

I179/SOGD

The Effect of Brimonidine Tartrate Ophthalmic Solution 0.2% (Alphagan®) on Pupil Size in Normal Individuals Under Different Luminance Conditions

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Aim: We evaluate the effect of brimonidine tartrate ophthalmic solution 0.2% (Alphagan®) on pupil size in normal individuals. Three different luminance conditions were used in order to assess the potential use of brimonidine in postoperative refractive patients who experience night time visual complaints related to large pupil size.

Methods: Pupil size was measured in 16 eyes of 16 participants with the Colvard Pupillometer (Oasis Medical, Glendora, CA, USA) under 3 different luminance conditions. One drop of Brimonidine 0.2% was administered to each patient. Pupil size was then measured using the same technique at 30 minutes, 4 hours and 6 hours after drop administration.

Results: Under scotopic conditions, 100% of the pupils showed significant miosis at 30 minutes ($P < 0.05$). The effect continued in all subjects for 4 hours. At 6 hours, a miotic effect was still present in 81.3%. However, under photopic luminance, all 16 eyes showed no significant effect on pupil size ($P > 0.05$). The pupil size of 5 eyes (31.2%) was not affected at 30 minutes, 4 hours or 6 hours. At 6 hours, 15 eyes (93.8%) had returned to their pre-instillation size.

Conclusion: Brimonidine 0.2% was shown to have a significant effect in decreasing pupil size under scotopic conditions. It can contribute to the reduction of night vision complaints, such as halos, starbursts, glare and monocular diplopia, as noted in postoperative refractive patients.

I180/SOGD

UCVA Intacs™ versus LASIK in Myopia Between -1.0D and -4.5D

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Aim: We compare the uncorrected visual acuity (UCVA) outcome of patients having Laser in-situ keratomileusis (LASIK) versus intrastromal corneal ring segments (ICRS) at day 1, day 7, month 1, month 3 and month 6 postoperative interval.

Methods: Forty eyes of 40 patients were compared; 20 eyes of 10 patients with Intacs™ and 20 eyes of 10 patients having LASIK. Patients' visual acuity was measured at day 1, day 7, month 1, month 3 and month 6; UCVA was reported and compared. Eyes were eliminated that did not have complete follow-up or were treated for monovision. Intacs™ that were removed or exchanged were eliminated, as well as any LASIK eye that had a complication that could affect best visual acuity.

Results: At the 20/40 and 20/20 or better UCVA level both Intacs™ and LASIK eyes had similar outcome. However, at 6 months, more than half of Intacs™ patients (65%) achieved 20/16 or better vision compared to only 5% of LASIK patients.

Conclusion: Both Intacs and LASIK are effective and safe for myopic patients between -1.0D and -4.5D. In this study, Intacs™ showed superior UCVA outcome at the 20/16 or better level at 6 months, while only 5% of LASIK patients achieved the same level. Intacs™ are superior with their ability to give better than 20/20 outcomes.

I181/SOGD

The Role of Myocutaneous Advancement Flap in the Prevention of Canthal Dystopia

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Aim: We describe a technique of lower eyelid reconstruction involving cheek and temporal myocutaneous flap dissection that minimises post-operative lateral ectropion and canthal dystopia.

Methods: The surgery involves the creation of a vertical temporal myocutaneous advancement flap (with attachments to the arcus marginalis) and lateral canthal resuspension. The medially advanced flap enabled the lower eyelid defects to be closed in layers without tension.

Results: Seven patients had lower eyelid defects corrected using this technique and attained stable lateral canthal angles with no lateral ectropion. The mean age of the patients was 74.1 years (range 49-87 years). There were 3 males and 4 females. Patients were followed up for a mean duration of 12.1 months (range 3-28 months). Reconstructions were performed after Mohs' excision of lower eyelid basal cell carcinoma (6 patients) and squamous cell carcinoma (1 patient). The lateral canthal skin incisions healed well, except in 1 patient with a slightly prominent scar. One patient had a lower eyelid wound dehiscence due to a long delay in eyelid reconstruction; the rest of the patients had uneventful postoperative healing without eyelid notching.

Conclusion: The myocutaneous advancement flap, with its periosteal fixation and vertical-temporal wound orientation, enables good support of the lateral eyelid and discourages lateral canthal dystopia.

I182/SOGD

Hydroxyapatite Orbital Implants: Indications, Changes in Surgical Techniques, Outcome and Complications

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Aim: We discuss the different indications for hydroxyapatite orbital implants, describe some modifications in surgical technique. we document the outcome and complications in a large series and compare the results with other published series.

Methods: Analysis of non-comparative series of 51 patients who had hydroxyapatite orbital implant operation in 1999 at Moorfields Eye Hospital and in 2000 at Cairo University Hospitals for different aetiologies. Data including history, examination, intraoperative findings, postoperative follow-up and complications were analysed.

Results: The mean age of the patients was 27 years (range 8-64). 45% of patients were males and 55% were females. The commonest indication for eye removal was trauma in 25 patients (49%). Orbital implant was done with evisceration in 15 patients (29%). Functional improvement was reported in 42 patients (82%). Postoperative infection was noted in 3 cases (6%) and exposure in 2 cases (4%).

Conclusion: Hydroxyapatite orbital implant is an effective way of treating orbital volume deficiency. It should be performed with some surgical modifications. Infection after orbital implant is the main cause of other postoperative complications.

I183/SOGD**Intacs™ (Intrastromal Corneal Ring Segments) Reversibility after Explant**Ahmed M EL-MOATASSEM*Ophthalmology, McDonald Eye Associates, Egypt*

Aim: McDonald Eye Associates, Fayetteville, Arkansas, USA is part of the KeraVision multicentre phase III study.

Methods: Data from 6 eyes, from which Intacs™ were removed, were evaluated with regard to segment size, loss or change of best corrected visual acuity (BCVA), any change in uncorrected visual acuity (UCVA), manifest refraction spherical equivalent (MRSE) and keratometric readings.

Results: Three eyes were explanted after 3 months, while the other rest were explanted at 6, 9 and 12 months, respectively. All patients were followed up for 6 months after the explant. They returned to their preoperative refraction level and had the same UCVA, MRSE and BCVA. The mean keratometric reading showed some flattening effect, indicating that the corneal curvature takes a longer time than refraction. However, this did not affect the visual end result or refraction outcome.

Conclusion: Intacs™ were easily and safely removed and the eyes returned to their preoperative refractive status within 6 months.

I184/SOGD**Monovision Outcomes with Laser in situ Keratomileusis (LASIK)**Seo Wei LEO¹, Hung Ming LEE¹, Siew Pang CHAN²*¹Department of Ophthalmology, Tan Tock Seng Hospital, Singapore, ²Division of Research and Evaluation, Health Promotion Board, Singapore*

Aim: We evaluate monovision outcomes after laser in-situ keratomileusis (LASIK) and identify factors affecting patient satisfaction with monovision LASIK.

Methods: A retrospective study of patients' demographic data, refractive outcomes, visual performance, satisfaction at various distances and under different lighting conditions was performed. Linear regression models were used to analyse factors related to satisfaction and to ascertain how post-operative visual acuities and spherical equivalents were associated with satisfaction (Stata 7.0).

Results: The mean satisfaction among 74 patients was 87.9%. The mean postoperative far and near vision spherical equivalents were -0.22D and -1.49D, respectively. Patients citing "inconvenience of spectacles/contact lens" as a reason for LASIK had significantly higher level of satisfaction. Satisfaction was not significantly associated with age, gender, occupation, religion, preoperative trial of monovision and visual acuity. Although a higher satisfaction level was recorded for patients with better postoperative unaided vision for distance, the result was not statistically significant. Similar observations were made in patients with good postoperative unaided near vision. All 17 patients with crossed monovision were satisfied.

Conclusion: Monovision is a viable option for presbyopes who want LASIK. Crossed monovision can result in satisfactory visual outcomes.

I185/SOGD**Paediatric Keratoplasty in Egypt: Indications, Visual Outcome and Complications in a Multicentre Study**Yassir ABOU-RAYYAH*Ophthalmology, Institute of Ophthalmology, University College London, UK*

Aim: We study the causes of potentially curable corneal blindness in children undergoing keratoplasty in Egypt and evaluate the visual outcome and complications of paediatric keratoplasty in Egypt.

Methods: A retrospective, non-comparative series of 78 children who presented to 3 centres within Cairo University Hospitals with corneal opacities of different aetiologies over a 2-year period (July 1995 to June 1997) was analysed. Case notes were reviewed for clinical and operative details and postoperative follow-up. Data were analysed using EPI INFO version 6.0.

Results: Penetrating keratoplasty was undertaken between 3 and 16 years of age. The main indication (diagnostic reason) for paediatric keratoplasty in the sample was scarring of the cornea due to post-microbial keratitis. This accounted for 51.3% of all keratoplasties. The next most common condition was trachomatous corneal scarring, which was responsible for 29.5% of the

cases. The preoperative visual acuity in the operated eye was very poor at hand movement or even worse in 68% of cases, and no better than 1/60 in the remaining children. The postoperative visual acuity in the operated eye at 6 months after surgery was 3/60 or better in 72.8% of cases. The main postoperative complication was graft rejection in 23.1% of children.

Conclusion: Paediatric keratoplasty is an effective method for treating corneal blindness in children in Egypt. More efforts should be directed at prevention of infective keratitis among children in Egypt.

I186/SOGD**LASIK for Unilateral High Myopia in Children**Ahmed MEL-MOATASSEM¹, Hassan EL-SAMMA², Ahmed Abdel-Basset SALLAM²*¹Ophthalmology, Ain-Shams University, Cairo, Egypt, ²Ain-Shams University Hospital, Cairo, Egypt*

Aim: We evaluate the safety, efficacy and predictability of using LASIK as a refractive surgical tool in children with unilateral high myopia, for whom conventional methods have failed.

Methods: Six eyes of 6 patients (3 boys and 3 girls) with high myopia and anisometropia (>3.5D) had the LASIK procedure to correct their vision. Best corrected visual acuity was measured before the procedure and at 2 months and 6 months post-procedure.

Results: All patients had at least 6 months of follow-up. The mean preoperative spherical equivalent refraction with cycloplegia was -8D (range -6.25D to -9D); it decreased to a mean postoperative spherical equivalent of -1.0D (range -2D to +0.5D) at 6 months. The preoperative visual acuity was <20/200 in all cases; the best corrected visual acuity ranged from 20/40 to 20/200. Half of our patients improved by at least 5 Snellen lines while the rest showed no improvement. None of the operated eyes lost any lines of its best corrected visual acuity. No patients gained any improvement in their stereopsis acuity.

Conclusion: LASIK can be performed safely and effectively in children who are compliant enough to undergo the procedure with topical anaesthesia. Refractive surgery does not improve vision in densely amblyopic eyes, but may give modest improvement in mildly amblyopic eyes. No significant complications were encountered other than a myopic shift over time.

I187/SOGD**Low-cost Intravitreal Ganciclovir Maintenance Injection for Cytomegalovirus Retinitis: Efficacy of an Intermediate Dose Regimen**Stephen Charn Beng TEOH, Ronaldo JARIN, Tock Han LIM*Department of Ophthalmology, The Eye Institute, Tan Tock Seng Hospital, Singapore*

Aim: We have previously reported the time to progression for cytomegalovirus (CMV) retinitis on weekly high-dose and low-dose intravitreal ganciclovir. This study measures time to progression for an intermediate-dose (1 mg/0.02 mL) regimen.

Methods: 34 eyes of 24 HAART-naïve patients with CMV retinitis received weekly 1 mg intravitreal ganciclovir. Progression was observed using photographic documentation.

Results: The mean time to progression was 608 days (95% CI 448, 768). The mean follow-up was 208 days. 3 eyes developed rhegmatogenous detachments, but none had endophthalmitis. Cost estimate was S\$750/eye/year (7.5% of sustained-release implant).

Conclusion: Weekly intermediate-dose ganciclovir is an efficacious option for patients who cannot afford implants.

I188/SOGD**A Comparative Study of Efficacy of Mesenchymal Stem Cells from Bone Marrow, Periosteum and Fat Transfers in the Treatment of Partial Growth Arrest**Yee Hong TEO¹, Li LI², James Hoi Po HUI¹, Eng Hin LEE¹*¹Department of Orthopaedic Surgery, National University Hospital, Singapore, ²Faculty of Medicine, National University of Singapore, Singapore*

Aim: We evaluate the efficacy of mesenchymal stem cells (MSCs) from bone marrow, periosteum, and fat in the treatment of partial growth arrest. In-vitro lineage differentiation was investigated and a rabbit model was created.

Methods: MSCs from bone marrow, periosteum and adipose were harvested and cultured. Various biological factors were used to induce chondrogenic and osteogenic differentiation. Calcium, alkaline phosphate activity and collagen II content were examined and compared. Medial half, proximal physis of tibiae in 6-week-old NZW rabbits were excised in our model. Cultured MSCs with fibrin glue (carrier) were transferred into the growth-plate defect following excision of bony bridge in established growth arrest. Contralateral tibiae received fibrin glue only as controls. 3 experimental groups of rabbits were established based on MSC source and were sacrificed at 8 and 16 weeks postoperatively. Clinical, radiological and histological analysis were performed.

Results: All MSCs demonstrated potential chondrogenic and osteogenic lineage differentiation ability. The proliferative rates were similar at days 4, 7 and 11. MSCs derived from the bone marrow and periosteum appeared more homogeneous than those from fat. All the tibiae treated with MSCs from the bone marrow and periosteum showed significant correction of varus deformity at 16 weeks. However, the varus angulation remained at 5.8° in the fat group. The limb difference with contralateral limb (control) in each animal was 0.65 ± 0.33 cm (bone marrow), 0.68 ± 0.24 cm (periosteum) and 1.22 ± 0.43 cm (adipose).

Conclusion: Bone marrow and periosteum MSCs better correct physeal arrest in rabbit. Hence, the source of MSC does influence treatment success.

I189/SOGD

Establishing an Osteoporosis Model for Tissue Engineering Application

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Aim: Osteoporosis and fragility fractures are projected to increase worldwide. With the myriads of tissue engineering techniques available, we believe bone regeneration via integration of mesenchymal stem cells (MSCs) into host cells is not impossible. We embark on a search for an osteoporosis model for our research.

Methods: 6-month-old female NZW rabbits were ovariectomised to induce osteoporosis. Both control (n = 6) and ovariectomised (n = 24) rabbits were fed normal diet and allowed to roam freely. We measured bone mass density (BMD), performed biomechanical testing (indentation test), histomorphometry (analysing 2-dimensional trabecular bone structures with an image analyser) and radiological analysis. Harvesting of MSCs was done by aspirating iliac crest of our rabbit; the yield was improved by mechanical stimulation and repeated puncture.

Results: BMD for ovariectomised rabbits decreased 3.5 standard deviation (318 ± 7 mg/cm³) versus increase in control. Less bone density, broader trabecular space and looser micro-architecture were seen on radiographs of the experimental animal. Histomorphometry revealed lower bone to total volume ratio of $22 \pm 9\%$ in ovariectomised rabbits versus $51 \pm 12\%$ in controls, lower trabecular thickness of $123 \pm 15\mu\text{m}$ vs $156 \pm 31\mu\text{m}$ (controls), lower nodes/free ends ratio of 0.53 ± 0.09 vs 0.98 ± 0.17 (controls). Ovariectomised rabbits achieved peak bone density 3 months after surgery before undergoing constant bone loss.

Conclusion: In our series, we have shown that NZW rabbit can be an effective osteoporosis model; the sizable bone mass with the presence of harvesian canals will most certainly supercede rodents as a model in such studies. Coupled with successful isolation and culture of MSCs from our rabbits, we are optimistic of conducting osteoporosis research at a cellular level with our model.

I190/SOGD

Unicompartmental Knee Arthroplasty: A Short-term Follow-Up

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Aim: Unicompartmental knee arthroplasty (UKA) was first introduced in the early 1970s, but its use is controversial from the start. Early initial results suggested that this new technique did not produce good enough results to

become a viable alternative to total knee arthroplasty. However, current studies have shown that in properly selected patients and with improved techniques, UKA could produce reliable favourable 8- to 10-year outcomes. UKA has also been shown to yield a higher initial success rate and had fewer complications. This has fuelled the resurgence in interest in the USA, Europe and Asia in the last 7 years.

Methods: We retrospectively analysed the records of patients who had UKA performed. All were operated on by the same surgeon. These patients were assessed pre- and post-operatively using the Hospital for Special Surgery Knee Disability Sheet for Knee Arthroplasty (HSS). Emphasis in data analysis was placed on pain, mobility and restoration of function.

Results: Ten patients (10 knees) were included in the study. The mean follow-up was 14 months (range 8 to 20 months). There were no perioperative complications and all were alive at follow-up. The preoperative mean HSS score was 54 and the postoperative score was 90. Range of motion improved from a mean of 70 to 110 degrees.

Conclusion: UKA has been shown to have distinct advantages. The incisions are smaller and patients have a shorter recuperation period. Bone stock and patellofemoral articulation are preserved. Results of UKA in this series are encouraging. These factors make UKA an attractive surgical option in patients with osteoarthritis.

I191/SOGD

Flap Coverage in Severe Open Injuries of the Lower Limb

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Aim: The aim of this study was to review the results of flap coverage in severe open Gustilo IIIB and IIIC.

Methods: Between 1999 and 2003, 37 lower limbs in 36 patients who sustained such injuries were treated at our institution. A total of 38 flaps were done. The cases were reviewed retrospectively.

Results: There were 32 IIIB and 2 IIIC fractures and 2 crush amputations of the forefoot. Twenty-eight patients were males. The mean age of the patients at the time of injury was 38 years (range 14 to 75 years). There were 27 injuries to the tibia, 3 to the ankle and 6 to the foot. The soft tissue defects ranged from 4 to 45 cm² in size. The time interval from injury to flap surgery ranged from 3 to 43 days (mean, 18.9 days). Twenty-eight were free flaps (12 gracilis, 10 latissimus dorsi and 6 rectus) and 10 were pedicled flaps (7 soleal, 2 gastrocnemius and 1 first metatarsal web). There was 1 failure involving free latissimus dorsi flap and 1 gracilis flap had partial necrosis. Of the 27 cases involving the tibia, 4 (14.8%) were nonunions. Preoperative wound cultures were positive in 36 samples taken from 24 patients, of which 25% were MRSA, 22.2% Ps aeruginosa and 13.8% Acinetobacter baumannii. Postoperatively, there were 3 cases of deep soft tissue infection.

Conclusion: Early flap coverage before colonisation of bacteria, appropriate antibiotic cover and early bone grafting are advocated.

I192/SOGD

Biliary Atresia and Liver Transplantation: The Singapore Experience

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Aim: Biliary atresia (BA) is a congenital malformation which, if untreated, leads to hepatic failure and death within 2 years of birth. It is the commonest indication for liver transplantation in children worldwide. The aim of the present study is to review the results of liver transplantation in children with BA at Singapore's national Liver Transplant Programme, which was set up at National University Hospital in 1996.

Methods: A retrospective review of paediatric patients with BA who underwent liver transplantation between 1996 and 2002 was conducted.

Results: During this period, a total of 33 children underwent liver transplantation. Of these, 25 had BA. Of these 25 children, 23 had undergone a prior Kasai's hepatic portoenterostomy. The waiting time for transplantation ranged from 5 days to 16 months, the median period being 2.6 months. A total of 27 transplant procedures were carried out in this group of 25 patients, with 2 patients requiring retransplants. The median age at transplantation was 1.7

years (range 1.1-14.2 years). 11 cadaveric and 16 living-related donor grafts were used. 20 (80%) patients remain alive, with follow-up periods ranging from 6 months to 7 years. Common post-operative complications included intra-abdominal abscess formation, bile leakage and portal vein thrombosis.

Conclusion: Our centre's experience with liver transplantation for children with BA has been encouraging. With the increase in cadaveric grafts expected due to changes in organ donation legislation, it is envisaged that more children suffering from BA will benefit from this procedure.

I193/SOGD

Management of Ventral Hernia Following Giant Exomphalos with External Pressure Compression Using Helmet Device

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Aim: Neonates with exomphalos major present a great challenge to paediatric surgeons. The abdominal cavity is small and underdeveloped. Primary surgical reduction of the herniated contents in such a case could result in compression of the liver, inferior vena cava, hepatic veins and decreased renal and pulmonary function. Hence, the initial management is conservative. The authors present a technique of controlled external compression using a specially constructed device.

Methods: Our patient is a full-term baby boy who was born with exomphalos major consisting of intestinal loops, with the liver protruding through a defect measuring 10 x 8 x 7 cm. No other major anomalies were detected. Controlled external compression using a specially constructed device was applied. Care was taken to avoid intra-abdominal hypertension by using the manometer readings and corroborated with observations of respiration, peripheral perfusion and urine output. The child was on full oral feeds and did not require any ventilatory support. Within 9 months, the treatment resulted in reduction of the exomphalos into a ventral hernia that was surgically repaired.

Results: The child did not demonstrate any ill effects of raised intra-abdominal pressure. Recovery was uneventful.

Conclusion: Application of controlled external pressure using a specially constructed device is an alternative method of conservative treatment when primary surgery is not possible. This device is safe, non-invasive, effective and can be constructed easily. The pressure applied is measured readily by directly reading the manometer attached to the cuff. However, the amniotic sac needs to be intact and the base of the sac must be large enough to allow uncomplicated reduction.

I194/SOGD

Quality Indices in a Cervicovaginal Cytology Service: Pre- and Post-Laboratory Accreditation

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Aim: Quality assurance practices contribute to the effectiveness of the cervical smear. Having been accredited by the College of American Pathologists (CAP) in 2000, we seek to identify changes in the quality indices of our cervicovaginal cytology service preceding and following accreditation.

Methods: Cervicovaginal cytology quality indices for 2001 (post-accreditation) were compared with those of 1997 (pre-accreditation). Performances in the CAP's interlaboratory comparison programme in cervicovaginal cytology (PAP) for the years 2000 to 2002 were analysed.

Results: A comparison between 1997 and 2001 shows a slightly higher inadequacy rate (1.3% versus 0.7%) in 2001, maintenance of a low ASCUS/SIL ratio (0.76 versus 0.82), a higher overall positive predictive value of a positive cytology (87% versus 79%) in 2001, fewer changes of the original cytological diagnoses in 2001 following review of significant cyto-histological discrepancies (2 versus 5 cases) and a higher subsequent positive yield of squamous intraepithelial lesions following ASCUS diagnoses in 2001 (41% versus 19%). The average discrepancy rate of the cytotechnologists and the laboratory consensus opinion in the PAP programme also showed improvement over the period 2000-2002 (2%, 1% and 0% [cytotechnologists] against 7%, 0% and 0% [consensus opinion], respectively).

Conclusion: An increased awareness of quality-related issues and participation in intradepartmental consultation and diagnostic seminars, all part of the accreditation process, have very likely contributed to the positive trends identified in the cytology service. Future challenges include increases in

workload, with the anticipated launch of Singapore's national cervical screening programme and adaptation to emerging cervical screening technologies.

I195/SOGD

Indocyanine Green Test: How Many Samples are Needed?

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Aim: The indocyanine green (ICG) test is used to evaluate hepatic function in patients undergoing hepatic resection. The procedure in our institution involves the collection of 6 timed blood samples (pretest, 0, 5, 10, 15 and 20 min). We examined how the procedure could be simplified while maintaining reliability of results.

Methods: The original laboratory records (serum absorbances) of all ICG tests performed between April 2002 and March 2003 were reanalysed using different mathematical models (1-point, 2-point and 4-point linear regression) and compared with the reported R15 (retention at 15 mins' post injection). The estimated retentions at time 0 was compared with the theoretical 100% to estimate overall errors in timing.

Results: 22 ICG tests were performed, of which 2 were invalid due to unlabelling/mislabelling of specimens. All mathematical models gave comparable answers. However, sample timing was out by ± 5 mins. Collecting a single specimen at 15 mins rather than a series runs the risk of an error in R15 of $\pm 12\%$. Reliability of results could be maintained with a reduction in samples from 6 to 4, recording of actual time of sampling rather than attempting to collect at specified times, and adjustment of the graph to give a 0-min retention of 100%.

Conclusion: Collection of blood samples for the ICG test can be simplified into 4 samples (pretest, 5-10 mins, 10-15 mins, 15-20 mins) with no loss of result quality. This is of benefit to both the patient and the clinician performing the test.

I196/SOGD

Repair of Calvarial Defects with Customised Tissue-Engineered Bone Grafts. Part I: Evaluation of Osteogenesis in a 3D Culture System

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Aim: We evaluate the morphology and osteogenic differentiation of bone marrow-derived mesenchymal progenitor cells and calvarial osteoblasts in a 2D and 3D culture environment.

Methods: NZ white rabbits were used to isolate osteoblasts from calvarial bone chips and bone marrow stromal cells from iliac crest bone marrow aspirates. Multi-lineage differentiation potential was evaluated in a 2D culture setting. Following amplification, the cells were seeded within a fibrin matrix into a 3D polycaprolactone (PCL) scaffold system.

Results: The constructs were cultured for up to 3 weeks in vitro and assayed for cell attachment and proliferation using phase contrast light, confocal laser, scanning electron microscopy and MTS cell metabolic assay. Osteogenic differentiation was analysed by determining the expression of ALP and osteocalcin. The bone marrow-derived progenitor cells demonstrated the potential to be induced to the osteogenic, adipogenic and chondrogenic pathways. In a 3D environment, cell-seeded PCL scaffolds evaluated by confocal laser microscopy revealed continuous cell proliferation and homogeneous cell distribution within the PCL scaffolds. Upon osteogenic induction, MPCs (12 U/l) produce significantly higher ($p < 0.05$) ALP activity than osteoblasts (2U/l); however, no significant differences were found in the osteocalcin expression.

Conclusion: This study showed that the combination of a mechanically stable synthetic framework (PCL scaffolds) and a biomimetic hydrogel (fibrin glue) provides a potential matrix for bone tissue engineering applications. Comparison of osteogenic differentiation between the 2 mesenchymal cell

sources revealed a similar pattern.

1197/SOGD

Shape and Contour Assessment of Asymmetric Human Mandibles Through 3D CT Image Analysis and Visualisation

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Aim: The aim of this project is to utilise 3D surface shape and contour assessment algorithms to develop a 3D analysis that will determine asymmetry of human mandibles.

Methods: Patients presenting with severe mandibular asymmetry which require surgical correction underwent computed tomography (CT) scanning at the National University Hospital as part of their treatment plan. CT data was obtained and analysed. The method involves image segmentation of the 3D CT image of the mandible from the craniofacial skeleton by double image thresholding. An algorithm is developed for the semi-automated detection of mandibular landmarks and subsequent establishment of the mandibular midline. Visualisation and 3D quantification of asymmetry are subsequently based on surface and contour matching algorithms and volumetric comparison between the left and right sides of the mandible.

Results: Our analysis enable us to identify the severity and origin of asymmetry. The area of aberrant or deficient growth is localised either in the ramal, condylar or body of the mandible or a combination. Our results confirm the clinical finding and, most importantly, allow surgeons to determine the directional vectors involved during distraction osteogenesis or the surgical target region during correction of the asymmetry. This information cannot be obtained from 2-dimensional cephalometric data or unprocessed CT data.

Conclusion: This 3D method of morphometric analysis permits clinicians to identify the anatomic location and to quantify the extent of anatomic asymmetry in the human mandible. It increases the accuracy and specificity of surgical planning for the correction of severe mandibular asymmetry and minimizes a second surgical intervention.

1198/SOGD

Delivery of Vascular Endothelial Growth Factor in Bone Regeneration: A Comparison of Vicryl and Polycaprolactone

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Aim: We compare the effectiveness of polyglactin 910 meshes (Vicryl, Ethicon, Somerville, NJ, USA) and custom made poly-caprolactone (PCL) as a scaffold for the delivery of vascular endothelial growth factor (VEGF) bone regeneration.

Methods: 36 adult New Zealand white rabbits (3.5- 4.0kg) with 72 parietal skull defects (10 mm in diameter) were used. 24 defects were grafted with scaffold (Vicryl or PCL) impregnated with rhVEGF (Calbiochem) and fibrin sealant (Tisseel®, Immuno AG) (Group 1). 24 defects were grafted with scaffold and fibrin (Group 2), and 16 defects grafted with scaffold alone (Group 3). 8 defects were left empty. An equal number of rabbits were sacrificed at 1 week and 2 weeks post-surgery and the tissue specimen harvested for histomorphometry analysis. The amount of new bone formed was quantified using the Olympus MicroImage software.

Results: At 2 weeks, significantly more bone was formed in defects grafted with Vicryl compared to PCL as a scaffold, in the presence of VEGF and fibrin (Group 1), fibrin only (Group 2) or used alone (Group 3).

Conclusion: Vicryl may be more effective than PCL in the delivery of VEGF for local application at bone defects in the short term, as observed in the rabbit model. Vicryl and VEGF may potentially be used as an effective tool to promote human bone repair.

1199/SOGD

Regeneration of Bone with Vascular Endothelial Growth Factor and Vicryl Mesh Scaffold

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Aim: We examine the osteogenicity of vascular endothelial growth factor

(VEGF) *in vivo* using fibrin sealant (Tisseel®, Immuno AG) as a carrier and polyglactin 910 mesh (Vicryl; Ethicon, Somerville, NJ, USA) as a scaffold.

Methods: 32 adult New Zealand white rabbits (3.5- 4.0kg) with 40 parietal skull defects (10 mm diameter) were used. 12 defects were grafted with Vicryl impregnated with rhVEGF (Calbiochem) and fibrin (Group 1). 12 defects grafted with Vicryl and fibrin only (Group 2) and 8 defects grafted with Vicryl alone (Group 3) formed the active controls. 8 defects that were left empty (Group 4) formed the passive controls. Equal number of rabbits were sacrificed at 1 week and 2 weeks post-surgery and the tissue specimen harvested for histological analysis. The amount of new bone formed was analysed using the Olympus MicroImage software.

Results: Defects grafted with Vicryl impregnated with fibrin and rhVEGF (Group 1) harvested at 2 weeks showed significantly more new bone formed compared to all control groups. Significantly more bone was formed at 2 weeks compared to 1 week in Group 1. Complete osteoid bone bridging across the defect was seen in Group 1 at 2 weeks, while all other groups showed only a little amount of new bone formed adjacent to defect margins

Conclusion: The responses to exogenous VEGF observed in the rabbit model indicated that VEGF, delivered with fibrin and Vicryl, applied locally at the site of bone defects did increase bone formation *in vivo*. This may potentially be an effective therapy to promote human bone repair.

1200/SOGD

Effects of pH on Surface Roughness of Glass Ionomer Restorative Materials

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Aim: The increased consumption of dietary acids has been linked to tooth surface loss, which may also affect dental restorations. This study aimed to determine the effect of pH on the surface roughness of commonly used glass ionomer restorative materials.

Methods: The materials evaluated were 2 highly viscous glass ionomer cements (Fuji IX and Ketac Molar). A compomer (Dyract AP) was used for comparison. Forty-two specimens (3 x 3 x 2 mm) were made for each material. The specimens were divided into 6 groups and conditioned in the following solutions at 37°C for 1 week: citric acid (pH 2, 3, 4, 5 and 6) and distilled water (pH 7). After conditioning, the surface roughness (Ra, µm) of each specimen was measured using a surface profilometer (SurfTest, Mitutoyo Corp., Tokyo, Japan). Data was analysed using 1-way ANOVA and Scheffe's test at a significance level of 0.05.

Results: The effects of pH on the surface roughness of glass ionomer restoratives were material-dependent. Ra values ranged from 0.04 µm to 0.15 µm and 0.04 µm to 4.4 µm for pH 7 and pH 2, respectively. The surface roughness of all materials evaluated was significantly affected by acids of low pH, with the glass ionomer materials being significantly rougher than the compomer at all pH levels.

Conclusion: The large increase in surface roughness of the highly viscous glass ionomer cements after exposure to acids may render them more susceptible to clinical failure.

1201/SOGD

Expression and Function of Chondroitin Sulfate on Palatal Wound Healing

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Aim: This study investigates the expression and function of chondroitin sulfate (CS) on palatal wound healing.

Methods: The expression of CS was observed by immuno-histochemistry technique in an *in vivo* wound healing model, which was made on the palatal mucosa of adult female New Zealand white (NZW) rabbits. The function of CS was investigated using the palatal fibroblast culture. Cells were treated with: 30 mM chlorate; 30 mM chlorate with 100 ng/ml CS or normal culture

medium. Cell adhesion and proliferation were quantified by MTS assay. Cell migration was observed in an *in vitro* wound healing model. One-way ANOVA was used for cell adhesion and proliferation.

Results: The expression of CS was increased in the wound repair region at the early healing stage and decreased to normal level 30 days after the operation. The chlorate-treated group of palatal fibroblasts exhibited fewer adherent cells at 4, 6, 8 and 10 hours ($p < 0.05$). Fibroblast proliferation was consistently slower in the chlorate-treated group at days 3 and 7 ($p < 0.05$). In the *in vitro* wound healing model to examine fibroblast migration, the wounded edges in the normal medium closed completely at 18 hours, and that of the chlorate-treated group were closed after 24 hours. The addition of CS can partially recover the inhibition effect of chlorate ($p < 0.05$).

Conclusion: CS was involved in palatal mucosa wound healing. *In vitro*, the addition of CS can partially recover the inhibition of chlorate on rabbit palatal fibroblast adhesion, proliferation and migration.

I202/SOGD

Development of a Robot for Skull-Base Surgery

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Aim: The NeuroBot system was developed to aid neuro- and otolaryngology surgeons achieve quick and precise drilling of the skull-base automatically. We aim to provide surgeons with a surgical planning station and a surgical navigation device by integrating these modules, which work interdependently, into the robotic system.

Methods: NeuroBot is made up of 4 distinct, but interdependent, modules. Fiducial markers are mounted on the skull. This patient marker system is subsequently registered and tracking of the patient and robot is done intra-operatively by the registration and tracking cum navigation system (NeuroRAT). The surgical planning workstation (NeuroVision) is used to combine computed tomography and magnetic resonance images, presenting the information in 3D to the surgeon. The surgeon pre-operatively defines the areas to be avoided and the system automatically generates the required path. The path is verified before instructions are sent to a base robot (NeuroBase), carrying a manipulator (NeuroPod). The NeuroPod is fitted with a surgical drill and a force sensor to monitor the drill sequence. The entire procedure is monitored by the surgeon through live video feedback and robot and patient tracking provided by NeuroRAT.

Results: Preliminary in-vitro tests have shown that NeuRobot could significantly reduce the amount of time spent on SBS with improved accuracy and safety.

Conclusion: This system greatly aids the surgeon in a complex surgery, such as SBS. Various modules of the system could be extended to adopt surgeon training and other surgical procedures requiring accurate and precise intervention.

I203/SOGD

Effects of Prophylaxis Regimens on Surface Roughness of Glass Ionomer Restorative Materials

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Aim: The aim of this study was to determine the effects of different prophylaxis regimens on the surface roughness of conventional (Fuji II Cap [FC], GC), resin-modified (Fuji II LC [FL], GC) and highly viscous (Fuji IX GP Fast [FN], GC) glass ionomer cements.

Methods: Five commonly employed prophylaxis regimens were selected for the study. They included brush with pumice-water slurry [PB], rubber cup with pumice-water slurry [PC], rubber cup with prophylaxis paste [PP], rubber cup with prophylaxis gel [PG] and air polishing [PJ]. Untreated specimens were used as controls. Eight specimens of each material were made for various treatment groups. For all groups, specimens were polished with 1200 grit grinding paper using a lapping device prior to treatment. Specimens

in the PJ group were subjected to 12 seconds of air-powder polishing. Groups involving the use of rotating devices were subjected to 12 seconds of treatment at a speed of 2000 rpm. Mean Ra [μm] was measured using surface profilometry. Data were subjected to ANOVA/Scheffe's tests at a significance level of 0.05.

Results: The range of Ra values for the different materials were as follows: FC - 0.30 to 1.70 μm ; FL - 0.40 to 2.51 μm ; and FN - 0.44 to 1.79 μm . For all materials, treatment with PJ resulted in significantly rougher surfaces when compared to the control group. For FC and FN, there is a significant increase in roughness after treatment with PB and PP, respectively.

Conclusion: Glass ionomer restoratives may require repolishing after treatment with some prophylaxis regimens.

I204/SOGD

Ability of Composite Core Materials to Mask the Colour of Pre-fabricated Posts under All-Ceramic Crowns

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Aim: The aim of this study was to evaluate the ability of composite core materials to mask underlying post when the recommended thickness of all-ceramic crowns was used.

Methods: Empress II disks ($n = 5$) of 1.2 mm thickness (shade A2) and 3 pre-fabricated posts (Aestheti-Post [A], C-post [C] and Parapost [P]) were selected for this study. Five composite disks of each material (Bis-core Natural® [BN], Bis-core Opaque® [BO], Corerestore2 White® [CW], Corerestore2 Universal® [CU], Core-Flo® [BCF] and Ti-Core Natural® [TC]) of 3 thicknesses (0.5, 0.75 and 1.0 mm) were fabricated. The composite disks were placed between the post and the ceramic disks and colour measurement was performed for all possible combinations using a spectrophotometer (Minolta, Japan). The combinations of ceramic and composite disks without posts served as control. CIE L a*b* were measured and Delta E values were calculated. The data were statistically analysed using one-way ANOVA with Tukey post-hoc test at a significance level of 0.05.

Results: The combination of 1.2-mm Empress II and 1 mm of all composite disks was able to mask the colour of all 3 types of post (Delta E < 2). When reduced to 0.75 mm, only BN, BO and CW showed Delta E values < 2 for all the posts tested. Most of the 0.5-mm disks were unable to mask the underlying colour.

Conclusion: The colour of the restorations was least affected by the colour of the post studied when a 1-mm thick composite core was placed under 1.2 mm of ceramic. BO showed the best masking ability with the lowest Delta E values, whereas BCF exhibited the highest Delta E values.

I205/SOGD

Surface Texture of Resin-Modified Glass Ionomer Cements: Effects of Finishing/Polishing Systems and Time

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Aim: We compare the effect of immediate/delayed finishing of resin-modified glass ionomer cements (RMGICs) on the surface roughness (Ra) of such restorations in the vertical (RaV) and horizontal (RaH) directions based on a tooth model.

Methods: Class V preparations were made on buccal and lingual/palatal surfaces of 64 freshly extracted teeth and restored with Fuji II LC and Photac-Fil Quick. Immediately after light polymerisation, gross finishing was performed with 8-fluted tungsten carbide burs. The teeth were then randomly divided into 4 groups of 16 teeth. Half of the teeth in each group were finished immediately, while the remaining half were finished after a week's storage in distilled water at 37°C. The following finishing/polishing systems were employed: Robot Carbides (RC); SuperSnap (SS); OneGloss (OG) and CompoSite Polishers (CS). The mean RaV and RaH (μm ; $n = 8$) were measured

using a profilometer.

Results: Data were subjected to ANOVA/Scheffe's tests and independent samples t-test at $p = 0.05$. In the immediate finishing groups, the mean RaV ranged from 0.59-1.31 and 0.83-1.52, while mean RaH ranged from 0.80-1.43 for Fuji II LC and Photac-Fil, respectively.

Conclusion: Choice of finishing systems is more critical in immediate finishing, with SS being preferred and CS as a viable alternative.

I206/SOGD

Non-destructive Characterisation of Dental Biocomposites Using Electronic Speckle Pattern

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Aim: The current international standard for determining the modulus of dental biocomposites involves the destruction of beam-shaped specimens. The aim of this study was to develop a non-destructive test for characterising the modulus of dental biocomposites.

Methods: Five visible-light-cured composites (Filtek A110 [AO], Z100 [ZO], Filtek Z250 [ZT], F2000 [FT] and Filtek Flow [FF]); 3M-ESPE were selected for this study. An electronic speckle pattern interferometry (ESPI) apparatus involving cantilever beam specimens (28 x 8 x 2 mm) was developed to determine modulus. Out-of-plane displacement upon force application was measured using 2 focus region lengths (22 mm [EI1] and 11 mm [EI2]) and the modulus ($n = 7$) was subsequently computed based on cantilever beam equations. Data was compared to those obtained from 3-point-bend flexural testing ($n = 7$) based on ISO4049:2000 specifications. Specimens were stored in distilled water at 37°C for 1 week prior to evaluation of all tests. Results were analysed using ANOVA/Scheffe's post-hoc tests ($p < 0.05$) and Pearson's correlation ($p < 0.01$).

Results: The modulus ranged from 5.53 GPa to 13.99 GPa, 5.78 GPa to 14.24 GPa and 4.26 GPa to 11.3 GPa for EI1, EI2 and ISO, respectively. For all 3 tests, the modulus of ZO and FT was significantly greater than ZT, which in turn was significantly greater than AO and FF. Correlation of EI1 and EI2 to ISO was significant, positive and very strong ($r = 0.94$ for EI1 and EI2).

Conclusion: ESPI may be a viable method of characterising the modulus of dental biocomposites. As it is a non-destructive test, time-dependent effects of composites can be determined using the same specimens, leading to substantial time and material savings.

I207/SOGD

Effects of Prophylaxis Regimens on Surface Roughness of Composite Restorative Materials

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Aim: The aim of this study was to determine the effects of different prophylaxis regimens on the surface roughness of microfill (Filtek A110 [AO]), flowable (Filtek Flowable [FF]) and polyacid-modified (F2000 [FT]) composites.

Methods: Five commonly employed prophylaxis regimens were selected for the study. They included (a) brush with pumice-water slurry [PB]; (b) rubber cup with pumice-water slurry [PC]; (c) rubber cup with prophylaxis paste [PP]; (c) rubber cup with prophylaxis gel [PG], and (d) air polishing [PJ]. Untreated specimens were used as the control group. Eight specimens of each material were made for the various treatment groups. For all groups, specimens were polished with 1200 grit grinding paper using a lapping device prior to treatment. Specimens in the PJ group were subjected to 12 seconds of air-powder polishing. Groups involving the use of rotating devices were subjected to 12 seconds of treatment at a speed of 2000 rpm. Surface roughness (Ra) was determined using profilometry and data was subjected to ANOVA/Scheffe's tests ($p < 0.05$).

Results: The range of Ra values for the different materials were as follows: AO - 0.09 to 2.17 mm, FF - 0.13 to 1.68 mm and FT - 0.42 to 1.25 mm. For

all materials, treatment with PJ resulted in significantly rougher surfaces compared to controls. Both AO and FF were also significantly roughened after treatment with PB. FT was significantly roughened after treatment with all prophylaxis regimens.

Conclusion: Composite resin restoratives may require re-polishing after treatment with some prophylaxis regimens.

I208/SOGD

Comparative Hardness and Modulus of Direct Tooth-coloured Restorative Materials

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Aim: The objective of this study was to compare the hardness and elastic modulus of the continuum of direct tooth-coloured restoratives.

Methods: Six restorative materials representing the continuum were selected for this study. They included an ormocer (Admira [AM], Voco), a giomer (Beautifil [BF], Shofu), a compomer (Dyract Extra [DE], Dentsply), a minifill composite (Esthet-X [EX], Dentsply), resin-modified (Fuji II LC [FL], GC) and highly viscous (Fuji IX [FN], GC) glass ionomer cements. Seven specimens were made for each material and stored in distilled water at 37°C for 1 month. Hardness and modulus of the materials were determined using depth-sensing micro-indentation testing. Multiple comparisons of the materials were performed using ANOVA/Scheffe's post-hoc test ($p < 0.05$). The correlation between modulus and hardness was determined using Pearson's correlation ($p < 0.01$).

Results: Mean hardness ranged from 46.44 to 72.65 HV, while mean modulus ranged from 7.86 to 12.77 GPa. The following significant differences were observed: Hardness – BF > DE, AM, FL and EX > FL; Modulus – FN > EX, DE, AM and FL, BF > AM. Correlation of micro-indentation modulus and hardness was significant and positive ($r = 0.55$).

Conclusion: Contrary to popular belief, the hardness and modulus of glass ionomer-based/containing restorative materials may be comparable or even superior to minifill and ormocer composites.

I209/SOGD

Effects of Thermal Fatigue on Mechanical Properties of Tooth-coloured Restorative Materials

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Aim: The objective of this study was to determine the effects of thermal fatigue on the modulus and hardness of direct tooth-coloured restoratives.

Methods: Five materials representing the continuum of tooth-coloured restorative materials were selected for this study. They included an ormocer (Admira [AM], Voco), a giomer (Beautifil [BF], Shofu), a compomer (Dyract Extra [DE], Dentsply), a composite (Esthet-X [EX], Dentsply), resin-modified (Fuji II LC [FL], GC) and highly viscous (Fuji IX [FN], GC) glass ionomer cements. Fourteen specimens were made for each material. The specimens were divided into 2 groups. Group A was stored in distilled water at 37°C for 1 month and Group B was thermocycled for 5000 cycles and stored for an identical time period. Hardness and modulus of the materials were determined using depth-sensing microindentation testing. Results were analysed using independent samples T-test ($p < 0.05$) and Pearson's correlation ($p < 0.01$).

Results: Hardness ranged from 46.44 to 72.65 and 49.11 to 78.97 HV, while modulus ranged from 7.86 to 12.78 and 8.12 to 13.13 GPa for Groups A and B, respectively. With the exception of BF, no significant change in hardness and modulus was observed for all materials. A significant increase in hardness was observed for BF specimens that were thermocycled. Correlation of modulus and hardness was significant and positive ($r = 0.56$).

Conclusion: Thermal fatigue does not change the mechanical properties of most direct tooth-coloured restorative materials. In view of this, the need for thermal cycling during biomechanical characterisation of these materials is questionable.

I210/SOGD**Long-Term Effect of Traditional and Alternative Intracoronary Bleaching Agents on Microhardness of Human Dentine**Hui Kheng CHNG¹, Adrian U Jin YAP¹, Hoe Boon ONG²¹Department of Restorative Dentistry, National University Hospital, Singapore, ²Department of Preventive Dentistry, National University Hospital, Singapore**Aim:** The purpose of this study was to determine the long-term effect of various bleaching agents on the microhardness of human dentine.**Methods:** Thirty-six human premolars were root canal-treated and randomly divided into 6 groups. Bleaching agents were sealed in pulp chambers as follows: group 1 (control) – water, group 2 – cotton pellet soaked with 30% hydrogen peroxide solution, group 3 – sodium perborate mixed with distilled water, group 4 – sodium perborate mixed with 30% hydrogen peroxide solution, group 5 – 35% carbamide peroxide gel and group 6 – 35% hydrogen peroxide gel. After 7 days, the teeth were sectioned and microhardness testing was carried out on inner and outer dentine. After storage for 3 months in distilled water at 37°C, microhardness testing was repeated.**Results:** There was significant reduction in dentine microhardness in groups 2, 5 and 6 immediately post-bleaching. There was significant further reduction in dentine microhardness in groups 1, 2, 3, 5 and 6 after 3 months of storage in water.**Conclusion:** Any decrease in microhardness of dentine post-bleaching was irreversible. Long-term storage in water results in further reduction in microhardness of dentine. Intracoronary bleaching with high concentration hydrogen peroxide should be avoided in cases with extensive loss of tooth structure, as the decrease in dentine microhardness associated with its use may predispose such teeth to fracture in function.**I211/SOGD****Effects of Food-simulating Liquids on Shear – Punch Strength of Dental Composite Restoratives**Sew Meng CHUNG¹, Mun Kit LEE², Adrian U Jin YAP¹, Chwee Teck LIM¹¹Department of Restorative Dentistry, National University of Singapore, Singapore, ²Division of Bioengineering, Faculty of Engineering, National University of Singapore, Singapore**Aim:** This study investigated the effect of food-simulating liquids on the shear punch strength of 2 composites (Tetric Ceram [TC], Vivadent and Esthet X [EX], Dentsply), a conventional (Compoglass [CG], Vivadent) composite and a posterior polyacid-modified (Dyract Posterior [DP], Dentsply) composite.**Methods:** Thirty-two specimens (10 mm in diameter and 1.6 mm thick) of each material were prepared. They were randomly divided into 4 groups of eight and conditioned for 1 week as follows: - Group 1 (control): air at 37°C; Group 2: distilled water at 37°C; Group 3: 75% ethanol-water solution at 37°C; and Group 4: heptane at 37°C. After conditioning, the specimens were restrained and subjected to shear punch strength testing using a 3.2-mm diameter punch at a crosshead speed of 0.5 mm/min. The shear punch strength of the specimens was computed and the data were subjected to ANOVA/Scheffe's tests at significance level of 0.05.**Results:** The effect of food-simulating liquids on shear strength was found to be material-dependent. All materials, with the exception of EX, were significantly weakened by ethanol solution. For DP, a significant increase in strength was observed after conditioning in water. EX was significantly stronger than TC and DY after conditioning in air, water and ethanol solution. The shear punch strength of EX and CG was significantly higher than DY after conditioning in heptane.**Conclusion:** The effect of food-simulating liquids on the shear strength of resin-based filling materials was product-dependent. Composites based on BisEMA may be more resistant to degradation by alcohol.**I212/SOGD****Comparison of the Physical Properties of MTA and Portland Cement**

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Aim: This study examined and compared the solubility, pH, and setting time

of ProRoot MTA (tooth-coloured formula) (WMTA), ProRoot MTA (original formula) (OMTA), White Portland (WP) and Ordinary Portland (OP) cements.

Methods: The pH of the cements as they set was measured with a temperature-compensated pH meter. Materials were tested for solubility by first immersing in distilled water for 24 hours, discarding the specimen and then evaporating the water and weighing the residue to calculate the solubility as a percentage of the original weight. The setting times were determined using Gillmore needles. All tests were conducted in accordance with ISO6876.**Results:** The solubility of the set cements was 1.28% wt for WMTA, 0.9721% wt for OMTA, 1.05% wt for WP and 1.06% wt for OP. The materials had average pH values of 12.78 for WMTA, 12.74 for OMTA, 13.07 for WP and 13.02 for OP. The setting time was 2 hr 20 min for WMTA, 2 hr 55 min for OMTA, 2 hr 15 min for WP and 2 hr 50 min for OP.**Conclusion:** The physical properties of all 4 cements tested were very similar. Further in-vitro and in-vivo tests should be conducted to determine the suitability of Portland cement for use as a cheaper alternative to MTA as a root-end filling material.**I213/SOGD****Clinical Application of Prognostic Factors for Patients with Organic Causes of Erectile Dysfunction on Sildenafil Citrate**

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Aim: There are still no clinical predictive factors to determine the response rate of erectile dysfunction patients (ED) to sildenafil citrate. The aims of this study were to evaluate and stratify the risk factors as well as to determine the prognostic factors in clinical practice to predict the response rate in order to improve the cost effectiveness and avoid side effects.**Methods:** This is an open label prospective study that includes patients who will be seen at the andrology clinic in 2 years' time. The patients were evaluated and investigated for possible underlying causes. ED severity was assessed by IIEF 5 with its duration and the degree and rigidity of erection. Psychogenic causes were excluded with a minimal follow-up of 6 months. They were all given 100 mg sildenafil citrate and were reassessed at the end of 6 months. Logistic regression with univariate and multivariate was used as the method of statistical analysis.**Results:** A total of 232 patients were in the cohort. The overall response rate was 43%; the best was seen in veno-occlusive cause and the worst in neurogenic cause. Age, smoking, diabetes mellitus, hypertension, hypercholesterolemia, pre-treatment IIEF, interval to achieve erection and duration of erection were significant factors in univariate analysis, but only age and smoking with IIEF were significant in multivariate analysis. With a combination of these factors, a table was centred to determine the response rate in clinical practice. This will assist physicians in selecting patients with a favourable response and to avoid unnecessary wastage of time and costs and to avoid side effect.**Conclusion:** Possible factors could be determined and used clinically to predict the response rate to sildenafil citrate.**I214/SOGD****The Use of Stretch Expanded Polytetrafluoroethylene as a Microvascular Graft in a Low-Flow Situation**Thien Chong WONG¹, Jane LIM², Thiam Chye LIM²¹Department of Surgery, National University Hospital, Singapore, ²Division of Plastic and Reconstructive Surgery, Department of Surgery, National University Hospital, National Medical Research Council, Singapore**Aim:** Microvascular reconstruction is often limited by the availability and length of the pedicle. The harvesting of autologous vein grafts adds morbidity and may not provide a good match. Expanded polytetrafluoroethylene (ePTFE) grafts have been used routinely in macrovascular surgery. However, there is only limited and somewhat inconclusive data on the performance of small-diameter PTFE grafts for pedicle lengthening in free flaps in a low-flow situation. In this study, we evaluated the efficacy of 3 mm-diameter stretch ePTFE grafts to lengthen the venous pedicle of a free flap.**Methods:** Five male New Zealand white rabbits were operated on under sterile conditions. Using an operating microscope, an epigastric flap was

raised and the pedicle was exposed and dissected to its origin from the superficial femoral vessels. A 2-cm segment of the vein was replaced with a stretch ePTFE graft. Anastomoses were performed using 9-0 nylon sutures. At 4 weeks postoperatively, the flaps were raised again with the pedicles re-explored. The graft was then removed and examined histologically.

Results: All the grafts demonstrated immediate patency and all the flaps were vital at 4 weeks postoperatively. When re-explored, the grafts appeared to be patent and covered by connective tissue. Light microscopy showed a thin fibrin layer covering the internal surface of the graft.

Conclusion: From this study, we conclude that 3 mm-diameter stretch ePTFE grafts can be used successfully as an alternative to bridging autologous vein grafts in the lengthening of free flap pedicles. However, longer grafts must be used to simulate clinical situations.

I215/SOGD

Repair of Calvarial Defects with Customised Tissue-Engineered Bone Grafts. Part II: Evaluation of Cellular Efficiency and Efficacy In Vivo

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Aim: We evaluate engineered cell-polymer constructs using calvarial osteoblasts and mesenchymal progenitor cells for reconstructing complex cranial defects.

Methods: 30 New Zealand white rabbits had bilateral parietal defects created. Based on computed tomography (CT) scans, customised polycaprolactone (PCL) scaffolds with precisely controlled microarchitecture were fabricated using rapid prototyping. Bone marrow-derived progenitor cells and osteoblasts were isolated and expanded in culture. Osteoblasts (Group I) and osteoprogenitor cells (Group II) were seeded with fibrin-glue suspension into 40 scaffolds. After incubating for 3 days, the scaffold-cell constructs and unseeded PCL scaffolds (control group) were implanted into 15-mm calvarial defects. Reconstruction was performed at 3 months.

Results: Osseous tissue integration within the implant and functionally stable restoration of the calvarium. Islands of early bone formation observed in X-rays and histological sections. Implants showed a high cellular-ECM ratio and dense vascular network. Mechanical testing: reconstructed area revealed partial integration with the surrounding cortico-cancellous bone. Amount of calcification measured using CT indicated cell-seeded constructs and measured about 60% more than unrepared/unseeded scaffolds. Mechanical investigations: stiffness reached 52 ± 29 MPa and 44 ± 16 MPa for MPC- and osteoblast-seeded scaffolds, respectively. Yield strength (push-out tests): 180 ± 36 N for normal calvarial bone, 90 ± 1 N (unrepared) and 106 ± 10 N (unseeded) constructs, which is about 60% of normal bone strength. MPC- and osteoblast-seeded scaffolds had yield strength of 149 ± 15 N and 164 ± 42 N, respectively, about 85-90% of normal bone.

Conclusion: This study demonstrated that biodegradable polymeric implants may be used to deliver osteogenic cells and enhance bone formation. Rapid prototyping technology produces scaffolds with controlled external geometry and microarchitecture, and offers new possibilities in the functional and aesthetic reconstruction of craniofacial defects.

I216/SOGD

Percussion, Diuresis and Inversion Therapy can Assist Passage of Lower Pole Renal Calculi after ESWL

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Aim: We compare the clearance rates of lower pole renal stones between

extracorporeal shock wave lithotripsy (ESWL) treatment alone and that of a combination of ESWL and mechanical percussion, diuresis and inversion (PDI) therapy on fragments after ESWL treatment.

Methods: 68 patients who underwent ESWL treatment for lower pole renal stones of total diameter ≤ 2 cm were prospectively randomised into 2 groups. The first group comprised 32 patients who received only ESWL treatment and the second group, 36 patients who had a median of 4 (range 1 to 12) sessions of PDI therapy about 1 to 2 weeks after each ESWL session. PDI therapy was performed with patients in a prone Trendelenberg position on a 45-degree angled couch, with at least 500 mL of oral fluids administered 30 minutes before therapy and continuous 10-minute manual mechanical percussion applied over the flank. Stone clearance was documented with plain abdominal radiographs at 1 month and 3 months following initial ESWL therapy.

Results: Patients from both groups were comparable in terms of mean age, total stone diameter, infundibular neck diameter, infundibular length, calyceal height and infundibular angles. All patients had a maximum of 3 ESWL treatments. Radiological complete stone clearance after 3 months for the ESWL alone group was 40.6% and that of the combination ESWL with PDI therapy group was 69.4%. This difference was statistically significant.

Conclusion: PDI therapy is useful in assisting passage of lower pole renal stone fragments after ESWL treatment.

I217/SOGD

Mandibular Fractures: Characteristics of Fractures and Results of Osteosyntheses with Titanium Implants Compared with Resorbable Implants in a Tertiary Hospital

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Aim: We attempt to establish the commonest aetiology for and sites of mandibular fractures and to compare the outcome of osteosyntheses with titanium to that with resorbable implants.

Methods: A retrospective analysis of 45 mandibular fractures with surgical interventions in our division from April 2000 to May 2003 was undertaken. There were 38 males and 7 females, 21 with open and 24 with closed fractures. All had open reductions and internal fixations of their fractures, except for 3 who had intermaxillary fixations. 4 of the 42 patients had osteosyntheses with resorbable implants; the rest had titanium implants.

Results: The commonest aetiology was road traffic accidents (55.6%) followed by falls (17.8%), industrial accidents (13.3%), assaults (8.9%) and sports-related accidents (4.4%). The commonest sites of fractures amongst a total of 74 fractures were parasymphiseal/symphiseal (36.4%) followed by angle (23%), condyle (18.9%), body (16.2%) and ramus of mandible (5.4%). The commonest complication encountered was chin paraesthesia/numbness, seen in 4 patients. 2 patients with open fractures developed osteomyelitis which required implant removal. All patients achieved fair to good occlusion after surgery, except for 2 patients who needed subsequent surgeries for malocclusion. No complications were encountered in the 4 patients with resorbable implants.

Conclusion: The commonest aetiology for mandibular fractures was road traffic accident and the commonest site of fracture was the symphyseal/parasymphiseal region. The use of resorbable implants did not result in a higher complication rate in this limited study.

I218/SOGD

Postoperative Pain Control after Laparoscopic Totally Extraperitoneal Groin Hernia Repair: A Comparison Between Continuous Bupivacaine Infusion versus Bolus Infusion

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Aim: Postoperative pain control after laparoscopic totally extraperitoneal/groin hernia repair (TEP) has elicited interest in recent years. It was thought that the enclosed extraperitoneal space after TEP would act as a reservoir for a bolus dose of bupivacaine. However, there have been mixed results with regard to postoperative pain control. We investigate a novel technique of continuous bupivacaine infusion using a catheter system and compare its efficacy with the bolus system.

Methods: 24 patients with groin hernia repair using TEP were randomised into 2 groups. At the end of the operation, group 1 received a bolus infusion of 40 cc of 0.25% bupivacaine in the extraperitoneal space, while group 2 had a catheter delivery system that delivered 0.5% bupivacaine at the rate of 2 cc per hour over 48 hours inserted into the same space. Pain scores were recorded at 6 hours and 24 hours postoperatively.

Results: The average pain score on the visual analogue scale at 6 hours for the bolus group was 34.5, with a minimum of 20 and a maximum of 40. The comparable values in the infusion group were 19.25, 0 and 30 ($t_{0.05} = 4.01$). The 24-hour record showed a still poorer pain control in the bolus group with values of bolus: Avg = 59, max = 90, min = 19; Infusion: Avg = 14.58, max = 20, min = 0 ($t_{0.05} = 3.03$) ($t > t_{0.05}$ in both). The patients with the infusion technique can also be sent home on the first postoperative day with earlier resumption of duties.

Conclusion: The infusion technique is superior to the bolus technique for postoperative pain control after laparoscopic TEP groin hernia repair.

I219/SOGD

Is There Any Benefit in Adding Breast Ultrasound to Standard Screening Mammogram for Breast Cancer?

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Aim: Ultrasound of the breasts is not part of the standard breast cancer screening protocol. This study examines if the addition of ultrasound to mammogram gives any useful added information that can affect patient management.

Methods: All patients with T1 and T2 tumours investigated in this hospital in 2001 and 2002 were included. 55 patients who had either mammogram or ultrasound, but not both, were excluded.

Results: n = 252. Age range: 24-88 years. Median age: 51 years. The majority were Chinese (86.5%). 70 (27.8%) patients had additional information on ultrasound. 19 (7.6%) patients had a second breast cancer found, of which 5 (2%) had multicentric and 14 (5.6%) had multifocal breast cancers. Mammogram and ultrasound correlated in 16 of these 19 cases, but in 3 cases (1.2%), the second tumour was detected only from the ultrasound. 17 (6.7%) patients had their breast lumps upgraded from benign to malignant based on additional ultrasound findings. 109 (43.3%) patients had no additional information on ultrasound. 40 (15.9%) patients had their tumours downgraded on ultrasound, but their management was still based on the standard mammogram.

Conclusion: Ultrasound added useful information in over a quarter of patients. Detection of second tumours by ultrasound, but not mammogram (1.2%), may affect these patients' surgical management in terms of breast conservation. Patients whose lumps were upgraded to malignant based on ultrasound may have had frozen section and margin control instead of excision biopsy during initial surgery. Thus, in a small proportion of patients, the addition of ultrasound can alter treatment.

I220/SOGD

The Interaction Between Cyclo-oxygenase and Inducible Nitric Oxide Synthase in Colon Cancer

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Aim: Cyclo-oxygenase (COX) and inducible nitric oxide synthase (iNOS) are 2 of the important inducible genes involved in inflammation and cancer. In this study, the interaction between these 2 genes in colon cancer cell lines was studied.

Methods: Three colon cancer cell lines (HCA7, HT29 and HCT116) with different COX-2 expression levels and activities were used to study the effect of S-nitroso-glutathione (GSNO), a NO donor. 7kb promoter sequence upstream of COX-2 gene was amplified and different COX-2 promoter plasmid constructs were used to evaluate which pathway NO affects to increase COX-2 expression. Cytokines' mixture can induce both COX-2 and iNOS expression. Different COX-2 or iNOS inhibitors were used to investigate if there were mutual regulatory effects between these 2 genes in colon cancer cell lines.

Results: GSNO increased PGE2 production and induced COX-1 and COX-

2 protein expression in all 3 cell lines in a dose- and time-dependent manner. Higher dosages of GSNO also inhibited cell growth and induced apoptosis, but this inhibitory effect is unrelated with the COX expression/activities of the cell lines because inhibition of COX did not further improve the inhibitory effect of GSNO on cell growth. Transient transfection analysis showed that GSNO did not increase the promoter activity of the 5' flanking region of the human COX-2 gene, which includes the NF κ B, IL-6 and CAMP response elements.

Conclusion: The inhibitory effect of NO on colon cancer cell lines is independent of their COX expression and activity. NO can stimulate COX protein expression and activity.

I221/SOGD

Implementation of Clinical Pathway Decreases Length of Stay and Hospital Charges for Patients Undergoing Breast Reconstruction

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Aim: Clinical pathways are designed to streamline patient care delivery and maximise efficiency while minimising cost. We evaluated the effect on cost and length of stay with the implementation of clinical care pathway for breast reconstruction patients. The average hospital stay for breast reconstruction pathway patients is 7 days.

Methods: Clinical pathway with the aim of improving quality of patient care was developed by a multidisciplinary team and implemented. The breast reconstruction pathway was implemented in November 2002 to standardise patient care. Data on length of stay and costs were collected for all patients undergoing breast reconstruction 1 year before and 1 year after pathway implementation. A total of 27 patients were included in the study. Total hospital charges and average length of stay were compared between 15 pilot patients not placed on the clinical pathway programme and 12 patients treated in the programme.

Results: The mean length of stay decreased from 6.9 days to 6.2 days for patients on pathway versus pilot patients. Mean hospital charges also decreased significantly, from S\$15,390 to S\$11,479 per patient ($P = 0.021$). Postoperative complications and rehospitalisation did not differ between the 2 groups.

Conclusion: With the implementation of the clinical pathway, average hospital charges and length of stay were reduced. The clinical pathway is considered a good tool in health care cost management. Additional research is needed to further assess the impact of clinical pathways on these patients.

I222/SOGD

Breast Reconstruction with the DIEP Flap: Our Experience of 45 Flaps

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Aim: The deep inferior epigastric perforator (DIEP) flap is now widely used as the first choice for autologous breast reconstruction. It was first described by Koshima and Soeda in 1989 and has replaced the transverse rectus abdominis muscle (TRAM) flap as the flap of choice in many centers. We evaluate the results of breast reconstruction with the DIEP flap in our patients.

Methods: A prospective study was undertaken using a standard database. Biographic data on the patients was recorded in each case with the operative details and postoperative course. Results were evaluated objectively and subjectively.

Results: 45 breast reconstructions were performed in 41 patients over a period of 3.5 years from July 1999 to October 2002. 4 patients had bilateral reconstructions while the rest had unilateral reconstructions. 32 patients had immediate reconstructions and 8 had delayed reconstructions. Well-known risk factors for free flap breast reconstructions were present: 7% were smokers, and 30% had abdominal scarring. There were 3 flap failures, with 6 partial flap failures. We achieved good results in 81% of our patients in terms of symmetry, projection and ptosis.

Conclusion: The DIEP flap, in our experience, is a reliable and safe technique for autologous breast reconstruction. It offers the patient the same advantages as the TRAM flap, with the additional benefit of preserving the rectus muscle. Donor morbidity and postoperative pain are reduced, leading to quicker recovery and reduced hospital stay. The more complex nature of this procedure is balanced by the gratifying results.

1223/SOGD

Breast Reconstruction: Local Experience

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Aim: Post-mastectomy breast reconstruction is performed more frequently due to improved patient awareness and availability of reconstructive options. This study compares the outcomes of the various methods used in our local experience.

Methods: Biographic data was recorded in each case with the operative details and post-operative course. Results were evaluated objectively and subjectively.

Results: Over the past 4 years, a total of 65 cases were performed using different methods. Autologous breast reconstruction was performed using the deep inferior epigastric perforator (DIEP) flap, transverse rectus abdominis muscle (TRAM) flap and latissimus dorsi flaps. The DIEP flap was popularised by Blondeel, Allen and Treece for breast reconstruction. It utilises the skin fat island of the TRAM flap without sacrificing the rectus muscle. We used the DIEP flap in 45, TRAM flap in 9, latissimus dorsi flap in 2 and breast implants in 9 of the cases. The failure rate was 6% for the DIEP flap group and 100% success for the groups who underwent TRAM and latissimus dorsi flap reconstructions. The DIEP flap group experienced less post-operative pain, quicker recovery and shorter hospital stay compared to the TRAM flap group. Generally, our patients were happy with the outcomes of reconstruction. The long-term results for the patients who underwent autologous reconstruction were better than the group with implants.

Conclusion: In our experience, the DIEP flap is a reliable and safe technique, with less donor morbidity. Nevertheless, with the many options available, the choice for breast reconstruction should be tailored to the individual.

1224/SOGD

Arteriovenous Fistula Using Transposed Basilic Vein

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Aim: When the cephalic vein is not available for a fistula at the wrist or elbow, the basilic vein is almost always of good size and quality, although too deep within the arm. If it is dissected and transposed into a new subcutaneous tunnel, it may be a useful fistula. In the UK, published results with this procedure are limited. We report our initial experience of basilic vein transposition over the last 3 years.

Methods: A retrospective review was performed of 11 patients who had basilic vein transposition. There were 4 males and 7 females, with a mean age of 53 (range 36-77) years. All patients underwent duplex scanning of the basilic vein. Nine patients had regional and 2 had general anaesthesia.

Results: There were no perioperative deaths. All 11 fistulae were technically successful, but 1 fistula needed thrombectomy. Nine of the 11 subsequently matured. One patient died before maturation. One fistula failed after 2 months. Of the 9 patent fistulae, 2 patients received renal transplantation. Two patients on dialysis died with functioning fistulae at 3 and 17 months. Four patients are having adequate dialysis at 6, 10, 10 and 19 months post-operatively. The remaining fistula is functioning, but has not yet been used for dialysis. Complications include superficial wound infection in 3 patients, 1 steal syndrome and 1 subclavian venous thrombosis.

Conclusion: Basilic vein transposition can be performed with acceptable patency and relatively few complications. It can prevent the need for synthetic grafts for formation of arteriovenous fistulae.

1225/SOGD

A Comparative Study of Ambulatory Circular Stapled Haemorrhoidectomy under Local Anaesthesia versus Circular Stapled Haemorrhoidectomy under Regional Anaesthesia

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Aim: The usage of circular stapled haemorrhoidectomy (CSH) has increased dramatically in recent years. Hitherto, it has been performed using regional or general anaesthesia. This study assesses the feasibility of performing CSH under local anaesthesia on an ambulatory basis and its acceptance by patients.

Methods: 60 patients with symptomatic 2nd or 3rd degree haemorrhoids were randomised into 2 groups. Group A had CSH under regional anaesthesia and were discharged on the following day. Group B had CSH under local anaesthesia and were discharged on the same admission day. Visual Analogue Pain Score was administered to both groups. In addition, Group B patients were asked questions regarding their satisfaction with the procedure.

Results: No significant differences in pain score and analgesic requirement were found between both groups of patients. All patients in Group B, except for 1, reported that they were satisfied to highly satisfied with their procedure.

Conclusion: CSH can be performed safely under local anaesthesia in an ambulatory care setting. The potential cost savings that may accrue would offset the cost of the stapler.

1226/SOGD

Congruence of Endoscopic and Histological Diagnosis of Gastritis

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Aim: Endoscopy is often used as an endpoint in the diagnosis of gastritis in spite of evidence showing a poor correlation between clinical diagnosis and histological congruence, both in terms of presence and type. We sought to achieve a consensus regarding the accuracy of endoscopic diagnosis and guidelines on the need for biopsy in all patients presenting for diagnostic upper gastrointestinal endoscopy.

Methods: We analysed retrospectively the OGD findings in 121 patients. The findings on endoscopy were based on the Sydney Classification with general intradepartmental consensus. Biopsies were obtained in all cases irrespective of findings. The findings were recorded as erythema, red spots, nodularity and atrophy, in addition to a specific "yes" or "no" answer for gastritis as a finding.

Results: Pain accounted for an overwhelming majority of symptoms (n = 84) followed by dyspepsia (n = 22) and bleeding (n = 16). Gastritis was noted on endoscopy in 63.6% (n = 77) of cases, while 36.4% (n = 44) of cases had normal findings. Of the 77 cases, 60 (77.9%) had histological confirmation, while only 9 (20.5%) of the other 44 had histological congruence. In generalised cases (pangastritis, n = 29), 69% (n = 20) matched histologically. An abnormal finding on endoscopy is about 77.9% sensitive, while a normal finding is only about 20.45% specific.

Conclusion: In the presence of obvious findings endoscopically, the correlation is likely to be very high, while negative findings are likely to be extremely fallacious. Biopsies must be taken in all clinically suspected cases.

1227/SOGD

The Missing Cleavage: A Report of a Case of Synmastia

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Aim: We report a rare case of synmastia in an 18-year-old female. The authors propose an original technique for correction of synmastia with loss of the intermammary sulcus. The technique consists of defatting a presternal "gutter" using liposuction and fixation of the skin by sutures transfixing the sternum and tied over a pad.

Methods: An 18-year-old Indian woman was referred to our clinic for correction of synmastia with loss of intermammary sulcus. Ultrasound and mammographic examination showed that the breasts were connected by fatty tissue. There was no other medical or family history of note. Physical

examination revealed the absence of an intermammary sulcus. An operation involving liposuction of the presternal area was proposed.

Results: There has only been 1 previous reported case of symmastia in the literature. In view of the Asian predilection for keloids, especially in the sternal region, any incision in this area should be avoided. Taking into consideration her age and the possibility of childrearing and breastfeeding, any options that disrupt the ductular or glandular tissue in either breast should be avoided. Several options have been considered in order to dissect the entire thoracic cutaneo-glandular tissue in the presternal region. Options may include transaxillary or transareolar endoscopic approach, bilateral perioareolar incisions with subcutaneous dissection, inframammary, subxiphoid or suprasternal approaches or a combination of the above.

Conclusion: We report a rare case of symmastia for which liposuction is proposed for the creation of an intermammary sulcus.

I228/SOGD

Factors Affecting Rate of Breast Conservation Surgery for Early Breast Cancer: An Observational Study in an Institution Between 2000 and 2002

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Aim: Breast conservation surgery (BCS) with post-operative radiotherapy is a well-established treatment for early breast cancer patients. This study attempts to find the factors affecting BCS in a local acute care hospital.

Methods: All stage I and II cases operated between 2000 and 2002, excluding T3N0 cases, were included in this study. Data was obtained from the breast cancer database and patients' casenotes.

Results: 389 cases were eligible for analysis. The mean age was 53.9 years (range 24 to 90 years). The racial distribution was similar to that in the local population. There was no difference in patient demographics seeing breast and non-breast subspecialists. In 25.7%, there was no documentation in the patient's casenotes that the BCS option was discussed (24% in breast surgeons and 30.6% in other surgeons, $p = 0.314$). Indications for mastectomy were more likely to be documented by breast surgeons (45%) than by other surgeons (30.6%) ($p = 0.052$). Univariate analysis showed that patients who had T1 tumours were operated on in 2002 and those operated on by breast subspecialists were more likely to have BCS. Multivariate analysis, after excluding patients with strong indications for mastectomy, showed that the year of operation and surgeon type remained independent predictors of BCS.

Conclusion: BCS in this institution is largely influenced by the surgeon's subspecialty training and tumour size. Other factors, including patient choice do not appear to influence BCS rates. The surgeon plays a crucial role in increasing the BCS rate in the treatment of early breast cancer.

I229/SOGD

Assessment of Biocompatibility of a Microporous Polyurethane Synthetic Analogue (NeuroPatch) in Reconstruction of Orbital Fractures

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Aim: Various synthetic analogues are available for use in reconstruction of the orbital wall after traumatic injury. They are used to cover defects in the orbital wall and to overlay reduced fracture sites to prevent soft tissue entrapment during healing. Consideration needs to be given to their ability to provide a solid support lattice during healing and to maintain post-reduction and fixation orbital volume (functional biocompatibility). One also need to consider the risk of developing host reaction with resulting inflammation and extrusion (constitutional biocompatibility). This study specifically examines the biocompatibility of NeuroPatch, a microporous polyurethane material.

Methods: Rates of persistent post-operative diplopia, enophthalmos, implant infection and extrusion are examined in a retrospective review of 105 patients who underwent orbital fracture reconstruction using NeuroPatch between October 1997 and December 2002. Patients were followed up for at least 1 month and the average duration of follow-up was 6.62 months. The complication rates were compared with that of various synthetic analogues in

other case series.

Results: The overall complication rate was 5.7%, with a 1.9% rate of persistent diplopia that did not resolve with conservative measures, 2.85% infection rate and 0.95% implant extrusion rate.

Conclusion: NeuroPatch compares favourably with other synthetic analogues in terms of diplopia and enophthalmos rate, but has slightly higher extrusion and infection rates. Taking study size and fracture subtype differences with other series into consideration, NeuroPatch remains a viable tool in orbital fracture reconstruction with good biocompatibility.

I230/SOGD

Patients' Expectation and Experience of Visual Sensations During Cataract Surgery Under Topical Anaesthesia

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Aim: To investigate what patients expect to see and the visual sensations they experience during cataract surgery under topical anaesthesia. We also sought to determine if patients find their intraoperative visual experience frightening and the factors that are associated with this.

Methods: One hundred and 1 patients (47 males and 54 females) who underwent phacoemulsification and intraocular lens implantation under topical anaesthesia were interviewed preoperatively on what they expected to see with their operated eye during surgery, and postoperatively on the visual sensations they actually experienced.

Results: Preoperatively, 39 (38.6%) patients expected some light perception during surgery, 38 (37.6%) patients expected no light perception and 24 (23.8%) did not know what to expect. Many patients who expected light perception also expected to experience a variety of visual sensations. Postoperatively, all patients reported that they saw at least some light intraoperatively and some saw a variety of visual sensations. Twenty (19.8%) patients found their visual experience frightening. Patients who were anxious, saw movement intraoperatively or did not experience a sudden increase in clarity intraoperatively were statistically more likely to be frightened.

Conclusion: A majority of patients either expected that they would not perceive light throughout their cataract surgery under topical anaesthesia or were unsure of what to expect. All patients experienced at least some light perception, and many perceived a variety of visual sensations. These were frightening to nearly 1 out of 5 patients.

I231/SOGD

Patients' Expectation and Experience of Visual Sensations during Cataract Surgery under Regional Anaesthesia

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Aim: To investigate and compare patients' expectation and experience of intraoperative visual sensations during cataract surgery under retrobulbar anaesthesia, and to determine whether they found their intraoperative visual experiences frightening and the factors influencing this.

Methods: One hundred and fifty-two consecutive patients undergoing routine phacoemulsification or extracapsular cataract extraction (ECCE), with posterior chamber intraocular lens implantation under retrobulbar anaesthesia, were interviewed peri-operatively regarding their expected and actual visual sensations in the operated eye during surgery.

Results: Seventy-two men (47.4%) and 80 women (52.6%) participated in the study. One hundred and 2 patients (67.1%) underwent phacoemulsification and 50 patients (32.9%) had ECCE. Forty-nine patients (32.2%) expected to be able to see at least some light during surgery. Thirty-two (21.1%) felt that they might be frightened by the intra-operative experience. Postoperatively, 127 patients (83.6%) saw at least some light. Many of those who saw some light also experienced a variety of visual sensations. Twenty-five patients (16.4%) reported no perception of light with the operated eye during the

entire surgery. Forty-five (29.6%) had transient no light perception. Thirteen patients (8.6%) were frightened by what they saw intraoperatively. Patients who had a longer operation ($P = 0.006$, multivariate analysis) were statistically more likely to find their visual experience frightening.

Conclusion: Many patients undergoing cataract surgery under retrobulbar anaesthesia experienced a variety of visual sensations even though most did not expect to see anything preoperatively. These sensations were frightening to some patients.

I232/SOGD

Compliance: Just How Important is This in the Use of Intranasal Steroids for Allergic Rhinitis

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Aim: Intranasal corticosteroids are recommended first-line therapy in adults with moderate/severe persistent allergic rhinitis (PAR). Discrepancies observed between treatment efficacy demonstrated in clinical trials and actual clinical practice could be a reflection of patient compliance. This study aims to evaluate the impact of patient compliance on the efficacy of treatment.

Methods: Sixty-three adult patients with moderate-to-severe PAR, and treated in an otolaryngology outpatient clinic with intranasal triamcinolone acetonate were randomly selected. Compliance was determined both by direct questioning and measurement of weight of medication consumed (WMC) after 4 weeks of therapy. Pre- and post-treatment nasal symptom scores were obtained.

Results: Less than 50% compliance was reported by 1 patient (1.6%) but detected in 7 patients (11%) by WMC measurement. Patients with lower WMC compliance were more likely to over report compliance on questioning. There was a significant ($p < 0.001$) improvement in the total symptom score before (7.45 ± 1.99) and after (3.59 ± 2.72) treatment in patients with at least 50% of WMC, but not when the WMC is $< 50\%$ (8.43 ± 1.72 vs 6.29 ± 2.43 , $p = 0.114$). The positive predictive values for each symptom relief (with $> 50\%$ compliance) were: itch = 70%, sneeze = 65%, running nose = 78% and blocked nose = 67%. The probability of therapy failure was inversely related to compliance by WMC.

Conclusion: Compliance has an impact on treatment efficacy. Patient history becomes increasingly unreliable as actual compliance falls. With the large discrepancy between reported and actual compliance, it is important to establish actual compliance when evaluating patients who failed to respond to therapy.

I233/SOGD

A Population Study of Nocturia in Singapore

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Aim: We estimate the prevalence of nocturia in Singaporean adults and assess its associations with sociodemographic and health factors.

Methods: In 1999, a door-to-door interview questionnaire survey was conducted amongst a randomly selected sample of 3000 individuals (response rate of 78.2%). Using questions from the International Prostate Symptoms Score (IPSS), nocturia and its associated problems were evaluated. Concurrently, sociodemographic and health parameters were recorded.

Results: The data of 1134 women (aged 20 to 95) and 1139 men (aged 20 to 92) were analysed. The overall prevalence of nocturia (1 or more voids/night, according to the International Continence Society definition) was 55.5%, with increasing numbers in the higher age groups ($p < 0.01$). Women had nocturia significantly ($p = 0.015$) more often than men (58% vs. 53%). It was positively associated with poor health, with the highest odds ratio for diabetes mellitus (OR = 2.0, CI = 1.3-3.1), renal disease (OR = 6.4, CI = 2.3-18.2) and strokes (OR = 3.1, CI = 1.1-9.2). Patients with previous major abdominal and pelvic surgery were also more likely to have nocturia ($p = 0.003$). In both men

and women, the median IPSS score in patients with nocturia was significantly higher than that in patients without nocturia ($p < 0.001$). 83% of respondents who woke up at least once did not consider it a problem at all, with 21% of them complaining of sleep and rest disturbances ($p < 0.001$).

Conclusion: Nocturia is a common symptom amongst Singaporean adults, especially in the elderly. It has strong associations with poor health and other lower urinary tract symptoms. In addition, nocturia is significantly linked to sleep disturbances.

I234/SOGD

The Safety Profile of Transrectal Ultrasound-guided Biopsy of the Prostate at a University Hospital

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Aim: Transrectal ultrasound-guided needle biopsy of the prostate is the procedure of choice for diagnosing prostate cancer. With current ongoing studies to evaluate screening programs for prostate cancer, this procedure will become increasingly widespread. We report on the experience and complications of this procedure at the National University Hospital.

Methods: Thirty-eight consecutive transrectal ultrasound guided needle prostate biopsies carried out in 2002 were audited retrospectively. The indications of biopsies, pre-operative preparation, ultrasound and biopsy results and complications were studied.

Results: The patients had a mean age of 67.5 (range 50-85) years. All but 2 of the biopsies were 10 core biopsies. The indications were elevated PSA leveling [22 (58%) patients], abnormal digital rectal examination [4 (10%) patients] or both [12 (32%) patients]. The median PSA level was 10 ng/ml (range 0.7-282.2). All patients complied with the pre-operative course of prophylactic ciprofloxacin and ducolax suppository. The mean prostate volume was 45.9 mls (range 14 to 121). A total of 10 (26.3%) prostate cancers were detected. There were no intraoperative complications, but post-operatively there were 2 (5.2%) episodes of mild haematuria, 2 (5.2%) episodes of fevers and 1 (2.6%) episode of urinary retention. None required hospital admission and all complications resolved with conservative treatment. There was no association of complications with prostate cancer or with lower urinary symptoms (19 patients) and high post-micturition volume (> 100 ml) (7 patients).

Conclusion: Transrectal ultrasound-guided needle biopsy of the prostate has an adequate safety profile for its widespread application in a population screening programme.

I235/SOGD

RPE65 Gene in India: A Phenotype Genotype Correlation in Retinal Degenerative Diseases

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Aim: We evaluate the clinical profile and correlate the RPE65 genotype of patients with retinal degenerative disorders.

Methods: Forty-six patients with Leber's congenital amaurosis (LCA) and early onset retinitis pigmentosa (RP) underwent detailed ophthalmic examination and electroretinography. They were subjected to pedigree analysis and mutation screening for the RPE 65 gene.

Results: Thirty-three (72%) patients were diagnosed with LCA and 13 (28%) had early onset RP. Thirty-five (76%) patients had onset of symptoms within 1 year after birth. Diffuse retinal pigment epithelial mottling was the most common fundus finding in LCA (16 patients, 48%), while typical bone spicule pigmentation, arteriolar attenuation and optic disc pallor were most common in RP (7 patients, 53%). Thirty (65%) patients had a history of consanguinity and first cousin unions constitute the majority (93%). Autosomal recessive inheritance was noted in 34 (73%) patients. Fifteen (32%) patients had nucleotide variations. One patient with RP had a homozygous mutation in exon 9 (N321K), while another patient with LCA had a heterozygous

mutation. Unusually, in 13 (28%) patients a silent mutation in exon 10 (E352E) was found.

Conclusion: There was no significant difference in the clinical presentation in patients with or without mutations in the RPE65 gene. Hyperopic refractive error and autosomal recessive inheritance were significantly associated with LCA ($p < 0.05$). RPE65 mutations reported in 16% of LCA and 2% of RP patients in the USA are not the common genetic cause of retinal degenerative diseases in India.

I236/SOGD

Clinical Profile, Risk Factors, Management, Microbiologic and Molecular Biologic Study of Endogenous Endophthalmitis in India

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Aim: To study the clinical, laboratory and management profile of endogenous endophthalmitis in India and correlate it with the risk factors.

Methods: A 5-year retrospective analysis of 70 patients (83 eyes) with a clinical diagnosis of endogenous endophthalmitis was done.

Results: Bilateral involvement was noted in 13 (18%) patients. Systemic predisposing conditions were noted in 44 (63%) patients. A history of intravenous infusions prior to presentation was the most common risk factor (25%). Majority of the eyes (36 eyes, 43%) had a diffuse involvement of both anterior and posterior segments. Cultures were positive in 51 (61%) eyes. Vitreous and aqueous were culture-positive in 55% and 18% of eyes, respectively. Bacterial and fungal infection was noted in 23 eyes each, while 5 eyes had mixed infection. The most frequent bacterial and fungal isolates were staphylococcus (34%) and candida (52%) respectively. Eye salvage was achieved in 76 (91%) eyes. Fundus view at the initial presentation had a better final visual outcome ($p = 0.003$, Pearson Chi Square test).

Conclusion: Fungal and bacterial endogenous endophthalmitis is equally common in culture-positive cases. Vitreous is a better sample for isolating the causative organism. Infusion of possibly contaminated intravenous fluids is the most significant predisposing factor noted for fungal endogenous infection in healthy individuals. Fundus view at initial presentation predicts a fair visual outcome. Despite a poor visual prognosis, eye salvage is possible in most cases.

Poster Presentation Abstracts – Day Two

I/II – Day One/Day Two; xxx – Poster Board Number; GP: General Practice; MP – Medicine/Paediatrics; NUR – Nursing; PD – Paramedical Disciplines; SOGD – Surgery/Obstetrics & Gynaecology/Dentistry

II001/GP

Patient Satisfaction: Through the Eye of QFD

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Aim: The health care industry is becoming a more and more challenging sector with hospitals constantly challenged to provide patients with high-quality and low-cost services. Specifically in Singapore this trend has been accentuated by an ageing population coupled with a change in the population health needs; rising expectations for better and more sophisticated healthcare services; and an increasing cost of providing healthcare. In this study, a new tool based on Quality Function Deployment (QFD) is attempted to address some of these issues.

Methods: The patient needs and expectations as well as all the healthcare features were first identified in correlation with inputs from staff of the National University Hospital (NUH). All these data were used to fill up the first House of Quality of the QFD process. A new multi-criteria optimisation was then applied and a set of Pareto-optimal solutions in terms of patient satisfaction and cost was generated.

Results: According to current trade-off preferences/constraints, factors contributing to the patient satisfaction were identified. It shed some light on the optimal level of quality improvement measures and their related costs for meeting a particular level of patient satisfaction. This helped in formulating guidelines and identifying opportunities for further enhancements.

Conclusion: The results only represent preliminary findings. They provide a test bed for the new QFD framework as a tool for formulating guidelines and policies for improving healthcare services. This could be a launching point for us to cope with the challenging Singaporean health environment.

II002/GP

Simplified Single-Tube Multiplex-PCR Screen for Anti-3.7 and Anti-4.2 α -Globin Gene Triplications

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Aim: The coexistence of α -globin gene triplication has been shown to be an important modulator of the severity of β -thalassaemia trait or β -thalassaemia intermedia, and screening to detect their presence is thus indicated for apparent β -thalassaemia carriers who have a more severe presentation than expected. Recent studies in Hong Kong and Sri Lanka suggest that the frequency of α -globin gene triplication carriers in these populations may range between 2-3.3%. Molecular screening for both the anti-3.7 and anti-4.2 α -globin gene triplications currently requires Southern blot analysis. We have developed a novel single-tube multiplex-PCR assay to screen for these gene triplications.

Methods: Anti-3.7/4.2 multiplex-PCR primers were designed to only amplify either an anti-3.7 or an anti-4.2 junction fragment from the respective triplicated allele (aaa). No amplification product would be generated from a normal allele (aa) or a deletional allele (-a or --). The amplicon sizes of the anti-3.7 and anti-4.2 junction fragments were designed to enable easy identification after agarose gel electrophoresis.

Results: The optimised assay was subjected to a double-blind validation analysis of more than 30 genomic DNA samples whose α -globin triplication status had previously been determined by Southern blot analysis. All tested samples were correctly genotyped, and the anti-3.7 and anti-4.2 alleles could be clearly distinguished. Test turnaround, including PCR and agarose gel analysis, was <6 h.

Conclusion: We have developed and validated a simple, rapid and reliable single-tube multiplex-PCR assay to detect the anti-3.7 and anti-4.2 α -globin gene triplications.

II003/MP

Antipsychotic Polypharmacy in Schizophrenia: Findings of the REAP (Research on East Asia Psychotropic Prescriptions) Study

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Aim: Previous studies on the prescription patterns of psychotropic medications in patients with schizophrenia have revealed a high rate of antipsychotic polypharmacy but the data in Asia are sparse. This multi-centre, international, cross-sectional study seeks to examine (1) the prevalence of antipsychotic polypharmacy in East Asia schizophrenia patients and (2) evaluate its clinical correlates.

Methods: Across 6 East Asian countries, territories and 32 centres, 2399 patients with schizophrenia were evaluated on their antipsychotic prescriptions. Daily doses of antipsychotic medications were converted to standard chlorpromazine equivalents.

Results: Antipsychotic polypharmacy was found in 46.4% (n = 1097) of the patients. Patients in Japan were most likely (OR 7.3, 95% CI 5.9-9.1, p<0.001) and patients in Hong Kong (OR 0.16, 95% CI 0.09-0.29, p<0.001) least likely to be in receipt of more than 1 antipsychotic medication. Polypharmacy was associated with male gender (OR 1.3, 95% CI 1.1-1.5, p<0.01), advanced age (t = -8.4, df = 2363, p<0.001), longer duration of illness (z = -8.29, p<0.001), psychopathology such as the presence of delusion (especially in Japan, OR 1.50, 95%CI 1.01-2.22, p<0.05), hallucination (OR = 1.19, 95% CI 1.01-1.40, p<0.05), aggression (OR 1.56, 95%CI 1.18-2.07, p<0.01), disorganised speech (especially in Korea, OR 1.53, 95% CI 1.02-2.30, p<0.05), adverse effects of constipation (p<0.001) and dry mouth (p<0.01) as well as higher daily CPZeq dosage (p<0.001), being on depot (p<0.001) and anticholinergic (p<0.001) medications.

Conclusion: The high rate of antipsychotic polypharmacy urges clinicians to constantly re-examine the rationale of such practice in light of recent postulates of possible benefits of combination therapy but not without its adverse effects.

II004/MP

Expansion and Differentiation of Mouse Bone Marrow Stromal Cells In vitro

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Aim: The adult bone marrow can potentially provide an abundant and replenishable source of stem cells for cell therapy. However, the culture conditions for growth and differentiation of bone marrow stromal cells (BMSCs) into cells of various tissue types have yet to be optimised and fully defined. This study was designed to explore the effects of various chemical reagents and conditioned medium on growth and differentiation of BMSCs.

Methods: We cultured and differentiated mouse BMSCs using various chemical reagents in combination with conditioned media from a number of tumour cell lines. Preliminary identification of differentiated cell types was based on microscopic observations. We are currently using immuno-histochemical and RT-PCR techniques to confirm and further characterise some of these differentiated cell types.

Results: Some of our differentiated cells were found to demonstrate a neuron-like morphology and exhibit positive staining for a number of neuronal markers.

Conclusion: This study shows that BMSCs can be differentiated into neuron-like cells in vitro. BMSCs can potentially provide an abundant source of cells for autologous transplantation in neurological diseases.

II005/MP

Modern Digital Echocardiographic Review: An Accurate and Safe Alternative to Videotaped Examinations?

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Aim: New digital storage of echocardiographic (Echo) data offers logistic advantages over videotaped archival. However, limited information is available on the accuracy of digitised examinations. We aimed to determine if this efficient review provides adequate diagnostic information without compromising patient (pt) management.

Methods: Transthoracic Echo images of 520 consecutive pts were prospectively acquired on videotaped and as digital cine-loops and still-frames. Two echocardiologists reported the digital followed immediately by the videotaped study. Using videotape as the gold standard, the significance of any Echo difference was graded from both imaging and clinical standpoints, and reasons for these differences identified.

Results: Irregular cardiac rhythms were present in 89 pts (17%). The complexity of Echo studies was low in 404 pts (78%), intermediate in 101 (19%) and high in 15 (3%). Echo images were of poor quality in 36 cases (7%). From an imaging perspective, differences between the images were deemed minor in 459 pts (88%), fairly significant in 55 (11%) and very significant in 6 (1%). The main reasons for the observed differences were: inadequate acquisition of views (59%), insufficient number of acquired cycles (25%) and suboptimal image quality (10%). Echo differences were considered to be of possible or definite clinical importance in 19 (4%) and 10 pts (2%), respectively. In a multivariate model incorporating rhythm, diagnostic complexity and image quality, the only independent predictor of significant difference between digitised and videotaped Echo images was study complexity ($X^2 = 17.1$, $P = 0.0016$).

Conclusion: The negative impact on clinical management decisions arising from exclusive modern digital review appears small. Greater attention towards acquisition of more complex studies should further minimise discrepancies between the 2 reporting methods.

II006/MP

All-Cause Mortality in Patients With Heart Failure and Left Ventricular Systolic Dysfunction

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Aim: To establish the 5-year all-cause mortality of patients with left ventricular systolic dysfunction admitted for heart failure.

Methods: 225 patients with left ventricular ejection fraction of 40% or less by echocardiography were admitted for heart failure in 1998. Five years later, the outcomes of these patients were determined via telephone. The primary outcome was all-cause mortality. Risk of death in relation to discharge medications and co-morbid medical conditions were also analysed

Results: Thirty-three (14.6%) patients were lost to follow-up. Of the remainder, 126 (65.6%) were dead and 66 (34.4%) alive after 5 years. Seventy-eight percent of patients in the cohort were treated with ACE-inhibitors. Patients were more likely to survive if they were on ACE inhibitors (RR of death 0.758, 95% CI 0.624-0.920, $p = 0.018$). There was a trend towards survival for those on statins (RR of death 0.796, 95% CI 0.604-1.048, $p = 0.072$). No survival benefit was observed with anti-thrombotic agents, calcium channel blockers, nitrates, hydralazine, or digoxin. In this cohort, only a small number of patients were on beta-blockers (6.3%) and spironolactone (6.3%). Patients in NYHA functional class 1 or 2 had a better outcome (RR of death 0.796, 95% CI 0.644-0.986, $p = 0.032$) than those in class 3 or 4, as did those who were younger than 70 years of age (RR of death 0.794, 95% CI 0.648-0.973, $p = 0.027$).

Conclusion: Mortality for this cohort of patients with heart failure was high despite the high usage of ACE-inhibitors. This highlights the need for a comprehensive heart failure management programme and evidence-based pharmacological therapy

II007/MP

Parent and Teacher Behavioural Ratings of Asian Children in Singapore

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Aim: To study the relationship between parents' and teachers' ratings of emotional and behavioural problems in school-going children in Singapore.

Methods: 270 children age 4-18 years were selected from children presenting at a child mental health facility in Singapore over a 3-year period. Each child was assessed by a psychiatrist who formulated a diagnosis, assessed the severity on a 3-point scale and completed a global assessment of function. Parents and teachers also completed behaviour checklists on the children's behaviour manifested within the past 6 months. Parent, teacher and clinician ratings were completed independently and their results analysed and compared.

Results: Analyses showed that parent and teacher ratings correlated positively and significantly ($r = 0.16$ to 0.35 , $p < 0.01$). Teacher ratings concurred significantly with clinician ratings on 7 behaviours while parent ratings concurred significantly with clinician ratings on 5 behaviours suggesting that teachers are better informants for screening children.

Conclusion: It appears that Singaporean teachers are good at picking up emotional and behavioural problems in children and may be helpful to clinicians in screening children for clinical assessment. The role of the school in detecting mental health problems can be a primary point for the prevention of mental illness in children.

II008/MP

High Frequency Ultrasound of Superficial Structures with a Water Bath

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Aim: High frequency linear ultrasound transducers allow detailed imaging of superficial structures. Reverberation artifacts from acoustic impedance mismatch can limit this use. We describe a technique that eliminates this artifact.

Methods: Five volunteers had an ultrasound examination of the small joints of the hands using a standard high frequency linear ultrasound transducer with a water bath. This was compared with conventional scans on the same volunteers using the same transducer and gel to ensure contact. Images of the metacarpophalangeal joints and proximal interphalangeal joints were obtained and compared using a 5 point scale.

Results: The reverberation artifact is absent from the images obtained using the water bath technique. There is better demonstration of the surface cartilage when the finger is flexed due to an improved acoustic window.

Conclusion: Use of a water bath technique is advantageous in the evaluation of superficial structures, especially when there is difficulty obtaining adequate contact with gel.

II009/MP

Association of Cyp2C9 Genetic Polymorphisms With Warfarin Requirement in a South-East Asian Population

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Aim: Warfarin, a common anticoagulant with narrow therapeutic window, is principally metabolised by the Cyp2C9 enzyme. Variant Cyp2C9 alleles may affect warfarin requirement.

Methods: Patients receiving maintenance warfarin with therapeutic INR underwent comprehensive Cyp2C9 sequencing.

Results: 102 patients participated, including 55 Chinese, 24 Malays and 20

Indians. Median warfarin dose was 3.5 mg, 3.5 mg, and 5.0 mg ($p = 0.001$) for Chinese, Malay and Indian respectively (range 1.0–8.0 mg, 1.5–6.0 mg, 1.5–11.0 mg). Eleven exonic, 5 intronic, and 33'UTR variants were identified. One Caucasian patient each was heterozygous and homozygous for the CYP2C9*2 allele (430C>T) that segregated with the (1582C>G) 3'UTR variant. Eight patients were heterozygous for the CYP2C9*3 allele (1075A>C), including 4 Chinese, 2 Malays and 2 Indians, that segregated with a silent variant (1425A>T, glycine>glycine). A novel nonsense mutation (485C>A, serine>termination) occurred in an Indian female. A 3'UTR frameshift mutation (1739delAT), occurred in a Chinese male. The other exonic variants were: 895A>G, 1144C>T, and 1361G>C, in 1 Chinese each, 374G>A and 1190A>C in 1 Indian each, and 208G>C in 1 Malay. Of note, the other previously described Cyp2C9 variant alleles, including exon 4 polymorphisms in Hong Kong Chinese, were not identified. The median warfarin dose for patients with Cyp2C9*3 was 1.5 mg, compared to 4.0 mg for wild-type.

Conclusion: The Cyp2C9*3 is present in Chinese, Malay and Indian, with allele frequency at 7.3%, 8.3% and 10.0%, respectively, and is associated with lower warfarin requirement. The Cyp2C9*2 allele is absent in Chinese and Malay. Multiple novel Cyp2C9 variants were found in our population that warrants further characterisation in an expanded cohort.

II010/MP

An Epidemiological Profile of Severe Head Injury Admitted to the National Neuroscience Institute (NNI)

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Aim: Severe head injury (SHI) is a common injury with significant high mortality and morbidity rate. This study aims to review the demographic and mechanism of injury of SHI in the National Neuroscience Institute (NNI).

Methods: This retrospective study was conducted in the NNI between January 1999 and December 2002. Information maintained in the Severe Head Injury Database which include demographic data, source of referral, details of injury, presenting Glasgow Coma Scale (GCS) and outcome at 6 months in 494 patients were reviewed.

Results: 79% of the SHI population are male and the age ranged from 10–96 years, with the young and active group (21 to 60 years old) constituting 67%. Common mechanisms of injury were motor vehicle (43%) and fall (47%) related accidents. 12% were under the influence of alcohol. 59% have GCS <8 on presentation and pupil abnormality was present in 34%. Traumatic SAH occurred in 33%, multiple injuries in 20% and radiological-proven cervical injuries in 10%. 44% were operated and the rest were treated conservatively. Average length of hospital stay was 23 days, with the maximum of 223 days. At 6 months post injury, mortality rate was 36%, unfavourable outcome (moderate to severe disability) in 14% and favourable outcome (slight or no disability) in 50%.

Conclusion: Motor vehicle and fall related accidents were the leading causes of SHI, and the patients were predominantly males and young. Half of these patients would be expected to have a favourable outcome.

II011/MP

Childhood Headache Referrals to the Paediatric Neurology Clinics in National University Hospital

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Aim: This prospective study aimed to evaluate the profile of children with primary headaches who were referred to the paediatric neurology clinics.

Methods: Questionnaires were completed by the children themselves or with the help of their parents. The characteristics of their headaches were used for diagnosis according to the criteria by the International Headache Society (IHS) established in 1988. The proposed modified criteria, according to the Paediatric Headache Committee of the Association for Study of Headache in 1997, were used for the diagnosis of paediatric migraine.

Results: There were 117 responders from January 1999 to May 2003. Using the modified paediatric IHS classification 29.9% had migraine without aura,

and 16.2% had migraine with aura, fulfilling the IHS diagnostic codes of 1.1 and 1.2 respectively. Episodic tension headaches (IHS 2.1) made up 19.6%; chronic tension headaches (IHS 2.2) 7.7%; and benign exertional headaches (IHS 4.5) 1.7%. The remaining 24.7% who were not either migraine or tension headaches were classified as "headaches of the tension-type, but not fulfilling IHS criteria" (IHS 2.3); "migrainous disorder not fulfilling criteria" (IHS 1.7) and "headaches not classifiable" (IHS 13.0).

Conclusion: As expected, the majority of patients referred to the subspecialty clinics have either migraine or tension-type headaches. However, we had significantly lower proportion of children who had migraine without aura when compared to reports from a tertiary centre in Barcelona, using the same modified IHS criteria for paediatric migraine. An epidemiological study would be useful to determine if Singapore children indeed have a lower incidence of migraine without aura.

II012/MP

The Community Screening Instrument for Dementia: Its Development and Validation as a Cognitive Screening Instrument for Use in the Three Main Ethnic Populations in Singapore

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Aim: The 10/66 Dementia Research Group recommended the CSID as a cultural and education-free cognitive screening instrument. This study aimed to develop and validate the CSID in the Chinese, Malay, and Indian elderly populations.

Methods: The CSID was translated to Mandarin, Malay, and Tamil and backtranslated, and assessed for acceptability and conceptual validity. The CSID, MMSE, AMT, and ECAQ, were administered to 84 Chinese, 85 Malay and 87 Indian elderly. All were independently diagnosed as demented or non-demented using DSM-IV criteria by a medical consultant.

Results: The CSID provides a cognitive score (CSID-cog), an informant rating of functional and cognitive decline (CSID-rel), and a combination score (CSID-df). Diagnostic accuracy of CSID-cog was high (0.97). It correlated highly with MMSE (0.90), AMT (0.88), and ECAQ (0.78). Accuracy was lower for CSID-rel (0.83) and CSID-df (0.92). For the Chinese, all 3 CSID scores had high accuracy (0.98, 0.93, and 0.96 for CSID-cog, CSID-rel, and CSID-df). For the Malays, accuracy of CSID-cog was at 0.98 and at 0.92 for CSID-df, but much lower for CSID-rel (0.74). Accuracy was at 0.95, 0.83, and 0.87 for CSID-cog, CSID-rel, and CSID-df for the Indians. Regressions analyses indicated significant contributions of race, age, and education to CSID-cog and CSID-df, and significant contributions of only race to CSID-rel.

Conclusion: The CSID appears to be a valid dementia screening instrument. Nevertheless, it remains influenced by educational and racial factors. Another interesting finding is that of racial differences in informant report of decline. Future studies to develop normal cutoff scores should take such factors into account.

II013/MP

MR Angiography of the Thoracic Great Vessels

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Aim: To demonstrate the feasibility of MR angiography to provide complete evaluation of the great vessels in the thorax and allow the medical and surgical planning of subsequent treatment.

Methods: Gadolinium MR angiography of the thorax was performed for 5 patients using a 1.5Tesla clinical MRI system and intravenous bolus infusion of gadolinium.

Results: Satisfactory demonstration of arterial and venous anatomy and pathology in these patients with 1) dissection of the thoracic aorta, 2) Takayasu's arteritis with occlusion of the proximal left subclavian artery, 3) occlusion of the superior vena cava and brachiocephalic veins, 4) occlusion

of the right and severe stenosis of the left internal jugular veins and 5) aberrant origin of the right subclavian artery with vascular ring demonstrated.

Conclusion: Gadolinium MR angiography allows non-invasive demonstration of arterial and venous anatomy and pathology in the thorax and can be performed as an outpatient procedure.

II014/MP

The Digital Future – The Digital Radiology Department Supporting eConsultation, eLearning, eTeaching and Facilitating Research

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Aim: To unlock the potential of the digital acquisition, processing, storage and distribution of radiological information.

Methods: To use current infrastructure to improve work processes with minimal additional monetary investment. The basis is DICOM-compatible imaging equipment connected to a departmental-wide PACS network, web-based hospital-wide image distribution, and creation of a web-based eLearning platform.

Results: We will report on the efforts to create a fully networked digital radiology department since 2001, followed by the delivery of images using a web-based filmless system and radiological report access using a hospital-wide electronic information access system. We will also report work in progress to create a web-based, interactive eLearning platform to facilitate medical student, medical officer, and radiology specialist trainee education, as well as subsequent continuing professional development.

Conclusion: The integrated application of eAccess, eConsult and eLearning strategies has the potential to significantly improve clinical service delivery and teaching, as well as support research and continuing professional development.

II015/MP

Utility of an Infrared Thermal Imager in Hyperthermia Screening in a Hospital Setting

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Aim: Severe acute respiratory syndrome (SARS) is a new infectious disease caused by a novel coronavirus. A high fever of greater than 38 degrees Celsius is essential in the case definition of SARS. Detection of individuals at higher risk of SARS, those with fever and a positive contact history are essential in minimising the chance of spread of disease within the hospital. Infrared thermal imagers have been proposed as an efficient and cost effective tool for screening the large number of individuals entering the hospital for hyperthermia. The authors present the results of a preliminary trial of 1 of these thermal imagers.

Methods: Tan Tock Seng Hospital (TTSH) evaluated a commercial thermal imager. The study took place at the triage station of the Emergency Department. The ear temperature of all patients was obtained using an infrared aural thermometer. The thermal imager, placed in front of the patient, obtained the maximum skin temperature reading of the upper half of the face. The 2 sets of readings were then analysed using receiver operating characteristic (ROC) curve analysis.

Results: The thermal imager threshold setting for 100% sensitivity for detection of a 37.5 degrees Celsius aural temperature, was 36.5 degrees Celsius. The readings from the thermal imager show good correlation with the ear temperature readings.

Conclusion: The thermal imager can be used as a first line tool for the screening of hyperthermia. The variation in the sensitivity of the imager can be addressed by the use of a recognised standard or calibration of the equipment.

II016/MP

MR Angiography of Pulmonary Atresia

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Aim: Pulmonary atresia is a rare congenital cardiac disease where the pulmonary valves do not develop. This results in an abnormal circulation where the pulmonary blood supply is achieved via major aortopulmonary collateral arteries (MAPCA). We describe the MR findings in children with pulmonary atresia.

Methods: Seven children patients had MR angiography on a 1.5 GE MR scanner for evaluation of pulmonary atresia as part of pre-operative assessment for MAPCA. A combination of pre-contrast T1, T2, single shot T2, flow sensitive gradient echo and contrast-enhanced T1 sequences in multiple planes was used. All patients were examined under general anaesthesia and cardiac or respiratory gating was used. All patients had extensive prior workup with obstetric ultrasound, neonatal cardiac ultrasound and cardiac catheterisation.

Results: There is great variation in the pulmonary artery and MAPCA. A residual pulmonary artery may be present and if so, its size is important in planning unifocalisation. The MAPCA can arise from anywhere in the thorax although usually from the descending thoracic aorta. Careful review of the source images and multiplanar reconstructions are needed to look for small MAPCA.

Conclusion: In young children with pulmonary atresia who are not suitable candidates for other investigations, contrast MRI is an alternative choice for accurate anatomical delineation prior to surgery.

II017/MP

Artifacts and Pitfalls in Chest Radiographs of Patients with SARS

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Aim: Severe acute respiratory syndrome (SARS) is a new infectious disease caused by a novel coronavirus. Chest radiographic findings of pneumonia are important in the reclassification of a suspect case to a probable case. The common initial chest radiographic findings are peripheral mid to lower zone ground-glass air-space shadows. Certain artifacts may mimic the subtle air-space shadows seen in SARS patients. The authors present the common artifacts on chest radiographs performed for SARS screening.

Methods: From mid March to late May 2003, more than 10 000 radiographs were performed for SARS patients and for SARS screening. The authors observed that several artifacts caused particular difficulty in the interpretation of these chest radiographs due to their similarity to the early findings in SARS.

Results: These artifacts were more common on the portable radiographs done for this group of patients as the procedure was technically more challenging compared to the conventional upright chest radiographic technique. The common pitfalls are patient rotation, suboptimal inspiration and prominent overlying soft tissue resulting in apparent air-space shadowing in the lower zones.

Conclusion: Familiarity with common artifacts and pitfalls in chest radiographs is important in making an accurate diagnosis of the common findings in a patient with SARS. Meticulous radiographic technique is helpful. Knowledge of pitfalls will help us avoid them.

II018/MP

High-resolution Computed Tomography Findings in Severe Acute Respiratory Syndrome in the Acute Phase and During Convalescence

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Aim: To describe the imaging features on high-resolution computed

tomography of severe acute respiratory syndrome both during the acute infection as well as during convalescence.

Methods: The high-resolution computed tomography studies of all probable severe acute respiratory syndrome patients in our hospital were reviewed retrospectively by 3 radiologists, individually and in consensus. The pattern of findings were noted and correlated with the duration between imaging and time of exposure to the virus.

Results: In the acute phase, the findings on high-resolution computed tomography are similar to other viral-induced pneumonias, showing a predominant peripheral and lower lobe involvement and progressing to an acute respiratory distress syndrome-type picture. A number of patients show evidence of interstitial fibrosis and scarring after recovery, only some of which is attributable to oxygen toxicity secondary to ventilator therapy.

Conclusion: High-resolution computed tomography is useful for the evaluation of severe acute respiratory syndrome, both in the acute phase and during convalescence. It may have a role in assessing the degree of residual respiratory functional deficit in patients who have clinically recovered from the disease.

II019/MP

Evaluation of CMV IgG on the Abbott AxSYM

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Aim: Cytomegalovirus (CMV) is a β -group herpesvirus, which causes oesophagitis, colitis, hepatitis, pneumonitis, renal tubulitis, chorioretinitis, and meningoencephalitis in neonates and in immunosuppressed persons secondary to transplant chemotherapy or AIDS. Currently all CMV IgG samples are sent off site. We evaluated CMV IgG measurement on the Abbott AxSYM, comparing the results to those from the offsite laboratory.

Methods: Imprecision was assessed using 2 levels of manufacturer supplied quality control materials over 20 days. Agreement between the Abbott AxSYM and the existing method was assessed using 45 routinely accepted patient samples.

Results: Imprecision results were: negative control: SD 0.52 AU/mL (level 0.26 AU/mL); positive control: SD 9.53 AU/mL (level 66.3 AU/mL). 45 samples were run for CMV IgG using the AxSYM system and the results compared with those from the offsite laboratory. 4 were negative by both methods while 41 were positive by both methods.

Conclusion: There is perfect correlation between the Abbott AxSYM CMV IgG assay and the existing service from the offsite laboratory. Provision of CMV IgG measurement onsite at TTSH should see both improved turn around time and cost benefits for the clinicians and patients of TTSH.

II020/MP

Emergency Physician versus Cardiologist Initiated Thrombolysis for Acute Myocardial Infarction: A Singapore Experience

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Aim: To compare the door-to-needle time between thrombolysis administration in the emergency department by the emergency physicians and those administered in the coronary care unit by the cardiologists.

Methods: Data were collected prospectively for all patients with ST segment elevation acute myocardial infarction (STEMI) who received thrombolysis in the emergency department over a 1-year period from January 2001 to December 2001. We recorded the time of arrival to the emergency department as well as the time thrombolytic therapy was commenced. These data were compared to the previous year, obtained retrospectively, where the patients received thrombolysis in the coronary care unit.

Results: The median door-to-needle time was significantly shorter in patients who were thrombolysed in the emergency department by the emergency physicians than in the coronary care unit by the cardiologists (29 minutes vs. 60 minutes, $p < 0.001$). There were no incidents of inappropriate thrombolysis nor were there incidents of major intracranial or gastrointestinal bleeding in patients thrombolysed in the emergency department. There was 1 case of medication dose error but there was no consequence to the patient.

Conclusion: Emergency physicians can administer thrombolytic treatment appropriately, quickly and safely in patients with acute STEMI.

II021/MP

Is World Health Organization Case Definitions for Severe Acute Respiratory Distress Syndrome Sufficient at Initial Screening?

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Aim: On March 13, 2003, Singapore doctors were alerted about an outbreak of atypical pneumonia that became known as Severe Acute Respiratory Syndrome (SARS). We describe a series of patients that did not fit the World Health Organization (WHO) case definitions for SARS at initial assessment.

Methods: The Ministry of Health, Singapore centralised SARS cases in the study hospital and its Emergency Department (ED) became the national screening centre. A screening questionnaire and a set of admission criteria based on the WHO case definitions were applied. A retrospective review was done for patients who did not fit these definitions initially, were discharged, then re-attended and were diagnosed to have SARS.

Results: For the first 8 weeks of the outbreak, 8,103 were screened for SARS. Among 7,218 (89.1%) discharged from ED, there were 28 re-attendees diagnosed to have SARS later, giving an under-triage rate of 0.4%. There were 6 (21.4%) who did not complain of fever and 22 (78.6%) had temperature $< 38.0^{\circ}\text{C}$ during their first ED visit. The initial mean temperature was 37.6°C (Standard deviation [SD] 0.8), which increased significantly ($p = 0.04$) to 38.0°C (SD 0.8) during their subsequent visit. Chest X-rays with infective changes increased significantly ($p = 0.009$) from 16% to 52.4% over the 2 ED visits.

Conclusion: The WHO case definitions were helpful in evaluating majority of SARS patients initially. However, under-triage at ED is inevitable with a 0.4% under-triage in our study population. In this group and asymptomatic individuals who came for screening, a tracking and recall system helped to ensure their timely return to the ED.

II022/MP

Heart Failure in Primary Care: A Singaporean Perspective

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Aim: Chronic heart failure (CHF) is a growing epidemic in Singapore. The diagnosis and management of CHF at primary care level is difficult. Misdiagnosis of CHF recently contributed to a community outbreak of Severe Acute Respiratory Syndrome (SARS). CHF remains the commonest cause of cardiac admissions despite advances in medical therapy, namely Angiotensin-Converting-Enzyme Inhibitors (ACEI) and beta-blockers. It is uncertain to what extent these advances have been implemented in primary care. We therefore seek to assess current practice patterns of general practitioners (GPs) and identify obstacles to optimal diagnosis and management of CHF.

Methods: Telephone interviews of 100 GPs using structured questionnaires.

Results: GPs diagnosed CHF based on the presence of breathlessness (92.5%), lung crackles (52.5%), peripheral oedema (55.0%) and findings on chest radiography (76.8%). Only one fifth mentioned raised jugular venous pressure as a useful diagnostic sign. Echocardiography is not commonly obtained (5%), mainly due to inaccessibility. While many (47.5%) were aware of the importance of ACEI, few (17.3%) knew of the impact of beta-blockers on morbidity and mortality. Instead, diuretics were thought to be most important for prolonging survival in CHF (47.5%). The main obstacle to widespread ACEI usage was dry cough. Patient compliance (57.8%) and cost (82.5%) were the main obstacles to optimising care.

Conclusion: This is the first study of primary care CHF practice patterns in Singapore. Local GPs diagnosed CHF using non-specific symptoms and signs. There was lack of access to echocardiography. Obstacles to optimal CHF management were identified. This will hopefully lead to strategies to improve patient care.

II023/MP

Severe Acute Respiratory Syndrome (SARS) in Patients on Chronic Peritoneal Dialysis (PD)

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Aim: The worldwide outbreak of SARS started in November 2002. SARS has not been reported in patients on chronic PD. The clinical features in renal failure patients are likely to be dissimilar to those without underlying comorbidities; and the respiratory function compromise may result in the need to terminate PD support.

Methods: We describe the clinical presentation and course of disease in 2 female PD patients with SARS as a result of contact with the index case between 2 March 2003 and 6 March 2003 at Tan Tock Seng Hospital, Singapore.

Results: The incubation periods were longer (13 and 14 days) as compared to 2 reports from Singapore (4-12 days) and Hong Kong (2-11 days). Patient A had fever that was classical of SARS. In contrast, Patient B did not mount any fever response throughout her admission. None had received immunosuppressive therapy. Patient A required ventilatory support. She did not develop Acute Respiratory Distress Syndrome (ARDS). Patient B had a self-limiting course, and required less than 50% supplemental oxygen. Patient A did not tolerate PD, which resulted in desaturation. She required intermittent haemodialysis from Day 5 to Day 15 of illness. She was recommenced on automated PD after Day 20 of illness. Patient B was able to tolerate PD throughout her entire course of illness.

Conclusion: End-stage renal failure patients are immuno-compromised. As such, the presentation of SARS in these patients may be atypical. The degree of respiratory function compromise will determine the renal replacement modality during the acute phase of the illness.

II024/MP

Some Observations on Prevalence of Primary Hyperaldosteronism in Hypertensive Patients in Singapore

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Aim: The prevalence of primary hyperaldosteronism (PHA) in hypertension has long been a contentious issue. It accounts for less than 1% of causes when only those with hypokalaemia are investigated. Majority of such patients have unilateral adrenal adenoma. Recent studies using PAC/PRA ratio suggest the prevalence of 5% to 15%. Such patients may have bilateral adrenal hyperplasia and many of them may have normal potassium. In 2000, Loh et al had conducted a study on patients attending 2 polyclinics to evaluate the prevalence of PHA in hypertensive patients in Singapore. We have attempted a similar study on patients attending a specialist centre.

Methods: 204 patients attending the outpatient clinics of General Medicine at Tan Tock Seng Hospital were selected. They were checked for random ambulatory measurement of PAC and PRA. Those with impaired renal function or on glucocorticoids or spironolactone were excluded. Screening was considered positive when PAC/PRA >20 and PAC >15 ng/dL. For the further confirmation of PHA, they were subjected to saline suppression test. In biochemically confirmed subjects, CT scans of abdomen were done to localise the tumour. In selected cases bilateral adrenal vein sampling was done to confirm abnormal aldosterone secretion.

Results: 41 (20.09%) patients were screened positive. 10 (28.7%) had biochemically confirmed PHA of which only 3 (33.3%) had hypokalaemia. CT abdomen and bilateral adrenal vein sampling revealed that half of them had evidence of unilateral adrenal adenoma.

Conclusion: The observations suggest that the prevalence of PHA in hypertensive patients at a specialist centre is around 5%, which is almost the same as that found in an earlier study conducted on patients attending the polyclinics.

II025/MP

Nephrotic Syndrome and Thrombotic Microangiopathy Associated With a Cyclooxygenase-2 Inhibitor

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Aim: Selective cyclooxygenase-2 (COX-2) inhibitors are a new class of anti-inflammatory drugs that decrease risk of gastrointestinal side effects commonly associated with conventional non-steroidal anti-inflammatory drugs (NSAIDs). Information on the renal toxicity of COX-2 inhibitors is scarce. We report the first case of thrombotic microangiopathy with nephrotic syndrome associated with the highly selective COX-2 inhibitor, etoricoxib.

Methods: A 63-year-old man developed nephrotic syndrome following ingestion of etoricoxib for left elbow tendonitis. He had no previous medical history and had acute renal failure on presentation. Serologies for rheumatological diseases were negative. The renal biopsy showed acute tubular necrosis, focal segmental glomerulosclerosis and renal thrombotic microangiopathy. There was extensive foot process effacement of the podocytes. He was treated with prednisolone but died 2 months later from pneumonia.

Results: Renal related adverse events have recently been reported with the use of celecoxib and rofecoxib. To date, there have been no reports of thrombotic microangiopathy associated with the use of COX-2 inhibitors. Considering the role of prostacyclin (PGI) in vasodilatation and platelet inhibition, the inhibition of renal prostacyclin by etoricoxib may predispose the patient to develop renal thrombotic microangiopathy. The extensive effacement of foot processes in the kidney biopsy supported the possibility of a drug-induced minimal change disease on a background of focal segmental glomerulosclerosis as the cause of nephrotic syndrome.

Conclusion: The renal adverse event profile of COX-2 inhibitors may be similar to NSAIDs. The same precautions should be used for this new class of drug as for non-specific COX inhibition.

II026/MP

The Use of Anticoagulants in Hospice — Who has the Tendency to Bleed?

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Aim: To determine the frequency of thromboembolic events and anticoagulant use. Risk factors predisposing to haemorrhage were also studied.

Methods: A retrospective study was done on 291 patients admitted to the Sacred Heart Hospice from July to December 2001. Logistic regression was used to calculate the odds ratio for the various factors. Forward stepwise regression produced the reduced model of the risk factors that predispose to haemorrhage in these patients.

Results: 42 (14.4%) patients had thromboembolic event within the last 1 year. Of these, 13 (31%) were given heparin and 11 (26.2%) were given warfarin. There were 59 (16.8%) episodes of haemorrhage amongst all patients, with upper gastrointestinal bleed being the commonest (32.2%). Anticoagulants, NSAIDs and a combination of the two were not significant risk factors for bleeding. Haematological disease (OR = 5.08), liver disease (OR = 4.44), occurrence of haemorrhage in the past 1 year (OR = 4.27), urinary tract malignancy (OR = 2.76) and ischaemic heart disease (OR = 2.55) were independent risk factors for bleeding. Peptic ulcer disease (OR = 4.12), diabetes mellitus (OR = 3.59), peripheral vascular disease (OR = 7.78) and occurrence of haemorrhagic episode in the past 1 year (OR = 4.33) were independent risk factors for upper gastrointestinal bleed.

Conclusion: This study presents an interesting finding — anticoagulants and/or antiplatelets were not significant risk factors for bleeding. This may be a reflection of the judicious use of these drugs. The risk factors identified will further help us to rationalise anticoagulant therapy for this group of patients.

II027/MP

Anaemia Management in End-stage Renal Failure Population

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Aim: Studies have shown that haemoglobin value below 11 g/dL in end-stage renal failure (ESRF) patients is associated with increased morbidity and mortality as compared to higher haemoglobin value. The target range for haemoglobin in ESRF patients according to American guidelines is between 11 g/dL and 12 g/dL.

Methods: We conducted a retrospective analysis of ESRF patients followed at Tan Tock Seng Hospital for the period between April 2002 and May 2003. Patients were included in this study if they have been on haemodialysis (HD) or peritoneal dialysis (PD) for at least 3 months. Patients were excluded if they had history of gastrointestinal bleed in the last 3 months and admission to hospital in the last 1 month. 68 HD and 30 PD patients (age, 27 to 87 years) were included in this retrospective analysis. Haemoglobin values, weekly erythropoietin dosages, transferrin saturations and serum ferritin concentrations were retrieved from the patient's medical records.

Results: 36.7% of the patients had haemoglobin equal to or greater than 11 g/dL; 32.7 % had haemoglobin between 9 g/dL and 10.9 g/dL; 29.6 % had haemoglobin below 9 g/dL; 11.2% of the patients had haemoglobin below 8 g/dL. Average weekly erythropoietin dosage is 3789 units. 80.4% of the patients had ferritin level >100 ng/mL and 87.2% of the patients had transferrin saturation >20%.

Conclusion: Despite sufficient iron stores, two-thirds of ESRF patients do not achieve the target haemoglobin, which is most likely related to inadequate erythropoietin dosage.

II028/MP

Catecholamines and Metanephrines Excess in Intracerebral Haemorrhage – A New Differential Diagnosis of Pheochromocytoma

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Aim: We postulate that any elevation of catecholamines (CATS) and metanephrines (METS) during the acute phase of intracerebral haemorrhage (ICH) should resolve, unlike true pheochromocytomas (PHEO). This study attempts to distinguish between ICH and PHEO by observing for temporary hypersecretions of CATS/METS in selected ICH cases.

Methods: 5 patients who satisfied pre-determined selection criteria were prospectively recruited. 24-hour urinary free catecholamines (U.CATS) and metanephrines (U.METS) were sampled on days 1 and 7 of hospitalisation, and before discharge.

Results: 4 out of 5 cases (80%) had pronounced elevations of U.CATS/METS into the ranges of PHEO (ie. > 2x upper limit of normal). The elevations of U.CATS/METS peaked towards the first week, followed by gradual taper over several weeks. Abdominal CT scans performed on the 2 patients with the highest levels of U.CATS/METS were negative for any adrenal or extra-adrenal masses. The levels of U.CATS/METS do not correlate well with the severity of hypertension. However, the magnitude of U.CATS/METS elevations was shown to be directly proportional to the size of the ICH and correlated to the site of ICH, being highest in frontal, followed by parieto-temporal and basal ganglia. U.CATS/METS was shown to be elevated much higher when ICH was complicated by intraventricular extension.

Conclusion: The transient pattern of U.CATS/METS elevations could allow ICH and PHEO to be distinguished. This may lead to lower utilisation of advanced imaging modalities and translates into substantial reduction in medical costs of investigations

II029/MP

Late Initiation of Dialysis in Chronic Renal Failure Patients

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Aim: Early initiation of dialysis before the development of uremic symptoms and malnutrition have been shown to improve patient outcomes compared with when dialysis is delayed until the glomerular filtration rate (GFR) is <5 mL/min or when patients have already developed signs of malnutrition.

Methods: We performed a retrospective chart review on end-stage renal failure patients who were initiated on dialysis at Tan Tock Seng hospital between February 2001 and January 2003. 108 haemodialysis and 30 peritoneal dialysis patients (mean age 62) were included in this study. Serum albumin and creatinine, as well as patient's age, gender and weight were retrieved from their medical records. The GFR was calculated using Cockcroft-Gault formula.

Results: Prior to initiation of dialysis, 31.7% of the patients had GFR below 5 mL/min, 50.8% had GFR between 5 and 10 mL/min, and 17.5% had GFR above 10 mL/min. The average GFR was 7.1 mL/min. 91.9% of patients had serum albumin below 40 g/dL and 37.8% had serum albumin below 30 g/L prior to initiation of dialysis.

Conclusion: One-third of the patients were initiated on dialysis once their GFR fell below 5 mL/min. The majority of the patients had signs of malnutrition before starting dialysis. Only a minority of these patients was initiated on dialysis before development of uremic complications.

II030/MP

Correlation Between Treatment Response to Electroconvulsive Therapy and Cytokine Levels

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Aim: To show a correlation between immunological changes in Interleukin-6 (IL-6) levels and patients clinical improvement following Electroconvulsive Therapy (ECT).

Methods: Informed consent was taken from 11 patients referred for ECT (6 diagnosed with schizophrenia, 5 with schizoaffective disorder). Serial blood samples were drawn before ECT and the centrifuged plasma samples kept at -70 degree Celsius. IL-6 levels were assayed using a commercially available ELISA. Each patient's response to ECT treatment was assessed using the Clinical Global Impression Scale.

Results: The 11 Chinese patients (5 males, 6 females), with a mean age 40.4 years (SD 9.7, range 21.9 to 54.7 years), had a mean duration of illness of 10.5 years. The concentration of IL-6 before ECT showed wide variation (mean 13.8 pg/mL, range 5.3 to 25.1 pg/mL). IL-6 levels after the final ECT (mean 11.6 pg/mL, range 4.2 to 23.1 pg/mL) were lower but the results were not significant. The CGI-improvement scores were between 1 and 2 at the final ECT and were positively correlated with the final IL-6 levels (Pearson Correlation 0.774 significant at the 0.01 level).

Conclusion: There have been few studies on the effects of ECT. ECT was thought to have an inhibitory effect on IL-6. This study confirms the changes in plasma IL-6 levels following ECT treatment. Although the difference did not reach significance levels, the IL-6 levels following the last ECT was positively correlated with clinical improvement in the patients' mental state.

II031/MP

Effectiveness of Oral Nutritional Supplements for Older Women With Hip and Other Fractures

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Aim: To assess the effectiveness of oral nutritional supplementation for undernourished older women with hip and other fractures, in improving

nutritional status, functional limitation, strength and morbidity.

Methods: Forty women over 70 years of age, hospitalised with hip or other fractures and whose mid upper arm circumference (MUAC) was less than 10 percentile for age and gender, or whose serum albumin was less than 35 gm/dL, were randomised to receive a daily additional oral nutritional supplement (201 kcal, 8.99 gm protein) for 40 days; or continue a normal diet. Demographic data, Barthel Index, Charlson Index, Short Portable Mental Status Questionnaire were also recorded. A research nurse, blinded to group allocation, assessed outcomes after 40 days and at 4 months after study enrollment. Primary outcome measures were mobility (as measured by the 2.44 metre walk) and nutritional status (MUAC). Complications and length of stay during the hospital admission were obtained from the subject's medical records.

Results: The groups were well matched in baseline variables. Significant improvement was seen in the intervention group at 4 month follow-up: (intervention group median, control group median) 2.44 metre walk (3.7 secs, 6.7 secs); MUAC (24.8cm, 21.5 cm); and grip strength (16 kg, 11.8kg). There is no difference in Barthel Index, or albumin (at 40 days).

Conclusion: There is a strong trend to benefit in the intervention group in nutritional and functional outcomes.

II032/MP

Predictors of Outcome after Hip Fracture

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Aim: Hip fractures cause significant morbidity and mortality in the elderly. To determine predictors of outcomes after hip fracture in a group of older persons admitted to a general hospital.

Methods: Retrospective chart review of all patients aged 60 years and older who were admitted for hip fracture from March to November 2001. Mobility status at 2-3 months post-discharge, post-operative complications, discharge destination, hospital readmission within 1 year of fracture and length of stay (LOS) in acute hospital. Chi-square statistics were used to identify factors that were significantly associated with the outcomes mentioned above (defined as $p < 0.05$). In addition, multinomial linear regression was used to ascertain predictors of LOS.

Results: 180 patients were studied. 25.6% of patients suffered a decline in their mobility status, with previous diagnosis of dementia and confusion on admission being significant associations. 28.3% of patients suffered post-operative complications. Female patients were noted to be more likely to have such complications. Additionally, residents of nursing homes and patients with dementia were also significantly more likely to have complications. 31.7% of patients were readmitted within 1 year. Significant associations included prior residence in nursing homes and pre-existent dementia. The mean LOS 16.8 $\pm$ 8.8 (3-66days). Factors predictive of LOS include the presence of complications, ischaemic heart disease, haemoglobin on admission, confusion on admission and patients requiring geriatrician review.

Conclusion: Older patients presenting with hip fractures are often complex. Patients with dementia and frail patients residing in nursing homes are more likely to have poorer outcomes.

II033/MP

Correlates of Habitual Walking and Sports/LTPA in Older Persons in Singapore: Interaction Effects between SES and Gender

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Aim: To determine demographic and psychosocial correlates on the participation of habitual walking and sports/leisure-time physical activity (LTPA) among older persons in Singapore.

Methods: In an observational study, 177 predominantly ethnic Chinese, community/urban-dwelling, ambulant, nondisabled participants age 50 years or older recruited from a health promotion programme. Main outcome measures were self-reported participation in habitual walking and sports/

LTPA. Variables examined in a multivariate linear regression analysis include socioeconomic status, demographic and health characteristics, social contact and health knowledge. In addition, interaction effects between gender and educational attainment were examined.

Results: The median age of participants was 63 years (range 50 to 81 years). Higher education attainment correlates negatively with participation in self-reported habitual walking ($p = 0.02$). Self-rated health and age influence sports/LTPA significantly ($p = 0.002$, $p = 0.012$ respectively). Those with better self-rated health and younger had higher participation in sports/LTPA than those with poorer self-rated health and older. In addition, the interaction between gender and educational attainment was important ($p = 0.019$). Men with higher education had a higher self-reported sport/LTPA; while in women, those with lower education attainment had a higher participation.

Conclusion: In contrast to prior findings from Western developed nations, previous studies done in Okinawa and Singapore (middle-aged women) found that SES and health behaviours may not be positively or directly associated. In this study, there is a negative correlation between educational attainment and participation in habitual walking and sports/LTPA especially among older Singaporean women. This suggests that older Singaporean women may have different characteristics in terms of social stratification or SES may have a different or non-direct effect on habitual walking and sport/LTPA self-efficacy.

II034/MP

Predominant Gastrointestinal Manifestations and High Symptomatic Ratio Among Adult Males in an Outbreak of Dengue Fever in Singapore

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Aim: The symptomatic ratio in an adult population has not been previously studied. In March-April 2002, we identified an outbreak of dengue fever involving 55 adult males of Chinese ethnicity who were epidemiologically linked to a construction site in Singapore. Their clinical information was prospectively collected and an epidemiological surveillance of the construction site was carried out to determine the attack rate and symptomatic ratio.

Methods: The clinical features and laboratory results of these 55 patients were presented and compared with results obtained from a previous study at our centre. Thirty days after the initial case, an epidemiological surveillance of symptoms and serum sampling were carried out involving 275 of workers living at the construction site. A virology study was performed to determine and compare the molecular sequence of the culprit virus with similar dengue subtypes isolated previously.

Results: They presented at a mean of 4 days (2-7) after the onset of symptoms and their median age was 34 years (21-41). Compared to a previous study of similar design, they had significant atypical features of nausea, diarrhoea, cervical lymphadenopathy, elevated urea and hypokalaemia. Epidemiologic surveillance study determined an attack rate of at least 14.7% and 100% symptomatic ratio. A dengue type 2 was isolated, the molecular sequence was similar to previously isolated strains in Singapore.

Conclusion: A significant gastrointestinal component was reported in this outbreak. A symptomatic ratio was reported for the first time in an adult population, unlike studies in children that showed a majority subclinical infection.

II035/MP

Evaluation of Total Cholesterol, Triglycerides, HDL-cholesterol and LDL-cholesterol on the Dimension RxL Clinical Chemistry System

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Aim: Clinical studies have shown that LDL cholesterol level is a key factor in the development of atherosclerosis and CAD and recommended as the primary determinant in diagnosis and treatment of hypercholesterolaemia. The Friedewald equations are still in used in estimating LDL-c despite its

several limitations. We evaluated these lipid parameters on Dimension RxL Clinical Chemistry System (Dade Behring Inc. IL) and compared with Cobas Integra 700 (Roche Diagnostics).

Methods: Dimension RxL uses enzymatic colorimetry to quantitate total cholesterol, triglycerides, HDL-c and LDL-c concentration by endpoint technique.

Results: Freshly collected sera were analysed where comparison studies showed that all lipid parameters correlated very well. Regression analyses obtained were 1) Dimension = 0.892 Cobas +0.306, $r = 0.992$ for cholesterol ($n = 126$); 2) Dimension = 1.073 Cobas +0.017, $r = 0.998$ for triglycerides ($n = 113$); 3) Dimension = 0.957 Cobas +0.036, $r = 0.983$ for HDL-c ($n = 111$). We have also carried out comparison studies for measured LDL-c and calculated LDL-c on Dimension analyser and found that the 2 assays are well correlated. Regression analyses were: measured LDL-c = 0.905 calculated LDL-c + 0.2256, $r = 0.969$ ($n = 100$) Total imprecision (%CV) of the lipids assays on the Dimension were determined. Cholesterol <1.2% (4.2 - 5.3mmol/L); triglycerides <2.3% (1.3 - 2.1 mmol/L); HDL-c <4.2% (0.8 - 1.01mmol/L). The total imprecision (%CV) for calculated LDL-c and measured LDL-c were also determined on the Dimension analyser. Calculated LDL-c <2.8% (range 1.5 to 4.5 mmol/L) and measured LDL-c <2.9% (range 1.5 to 4.5 mmol/L)

Conclusion: We concluded that Automated Dimension RxL Clinical Chemistry System is suited for routine lipid testing in a busy hospital clinical laboratory.

II036/MP

Comparison of the Greiner VacuetteR Serum Plastic Tube and BD Vacutainer SST II Serum Plastic Tube to the Vacutainer Glass Plain Tube

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Aim: Many clinical tests use anti-coagulant-free plain tube for specimen collection. VacuetteR (Greiner, bio-one) and Vacutainer SST II serum gel tube (Becton-Dickenson, US) contain a polymer barrier allowing immediate serum/clot separation after centrifugation. Hence simplifying work process and reducing turnaround time. Routinely measured analytes were evaluated against Vacutainer glass plain tube (Becton-Dickenson, US) for stability and any potential interference due to the barrier gel.

Methods: 35 healthy volunteers' blood was collected in 3 respective tubes. Prompt separation and analysis were done on Vacutainer glass tube, while the gel tubes were allowed to stand for 6 hours before analysis. 12 patients' sera with critical values for glucose, potassium, sodium and total calcium were collected and kept in both gel tubes for 6 hours then analysed. Results were compared to the original readings.

Results: Both gel tubes showed excellent correlation with glass plain tube for the following 31 analytes: sodium, potassium, chloride, urea, CO₂, creatinine, glucose, uric acid, total calcium, phosphorus, conjugated and unconjugated bilirubin, total protein, albumin, GGT, ALP, AST, ALT, LDH, CK, C-reactive-protein, CKMB, troponin-T, TSH, free-thyroxine, cholesterol, HDL-cholesterol, triglycerides, ferritin, transferrin and iron. 84% of the evaluated parameters have coefficient (r) values ranging from 0.90-1.00 over the reference ranges for healthy individuals. Patient samples showed $r^2 = 1.0$ (± 0.005) in both gel tubes.

Conclusion: The stability of the tested analytes in both VacuetteR and Vacutainer SST II tubes compares favourably to the Vacutainer glass tube. The stable inert gel barrier does not cause any adverse effect when immediate analysis is not possible.

II037/MP

Correlation of Anti-HCV Screening Reactive Results with Specific Recombinant Immunoblot Assay (RIBA) Results

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Aim: All anti-HCV screening (Chemiluminescence Immunoassay, Ortho-Clinical Diagnostics) reactive results tested in NUH, Immunoassay Laboratory are confirmed using a more specific Recombinant Immunoblot

Assay (RIBA, Chiron Corporation, USA). This is to ensure accuracy in interpretation and eliminate false positive results. Our study aims to determine the anti-HCV screening Cut-Off Index (COI) value predicting the RIBA result.

Methods: We reviewed 630 specimens tested as reactive for anti-HCV screening and their corresponding confirmatory RIBA results, from the period of January 2000 to May 2003.

Results: We studied RIBA results of anti-HCV screening reactive samples and grouped according to their COI values. The proportion of RIBA-positive samples with COI 1.0-2.0 was 5.8%; 2.0-5.0 was 8.3%; 5.0-10.0 was 18.7% and >10.0 was 92.6%. Grayzone reactive samples ($n=20$) with COI 0.9-1.0 were tested as RIBA negative. RIBA-indeterminate samples with COI 0.9-1.0 was 15%; 1.0-2.0 was 31.1%; 2.0-5.0 was 45.3%; 5.0-10.0 was 56.3% and >10.0 was 6.1%. RIBA-negative samples with COI 0.9-1.0 was 85%; 1.0-2.0 was 63.1%; 2.0-5.0 was 46.4%; 5.0-10.0 was 25% and >10.0 was 1.3%.

Conclusion: We observed a sensitivity (100%) and specificity (24%) of anti-HCV screening results (cut-off COI at 0.99) using RIBA results as reference. The poor specificity of screening results indicates the value of RIBA confirmatory testing. By verifying the presence of anti-HCV to get the correct diagnosis of patients infected with Hepatitis C virus, we can minimise the unnecessary medical costs to patient.

II038/MP

Anti-Glomerular Basement Membrane (GBM) Antibody Associated Glomerulonephritis: 5-year Single Centre Experience

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Aim: A retrospective analysis of anti-glomerular basement membrane (GBM) antibody associated glomerulonephritis.

Methods: All renal biopsies carried out from January 1997 to May 2003 were reviewed. Patients with biopsies that showed glomerulonephritis with linear IgG deposit in basement membrane on immunofluorescence microscopy were selected for study. Patients with lupus or IgA nephropathy were excluded.

Results: The ages of the patients were 41, 71, 52 and 40 years with serum creatinine at presentation were 456, 744, 857 and 1994 $\mu\text{mol/L}$, respectively. One patient had anti-MPO (p-ANCA) antibody. The patients presented with non-specific symptoms, e.g. lethargy, body ache, hypertension, and 1 patient with bilateral uveitis and all had severely impaired renal function requiring dialysis. Two patients were treated with corticosteroids, 1 of whom with intravenous cyclophosphamide and plasma exchange as well. Patients were followed up for 4 to 42 months. One patient (on dialysis) died of gram negative septicaemia after 12 months. Three patients are stable on dialysis. One patient presented after 2 months with pulmonary haemorrhage. He was managed with plasma exchange and intravenous cyclophosphamide. He is now on monthly cyclophosphamide therapy. At his initial presentation he developed steroid related perforated duodenal ulcer. None had cyclophosphamide-associated complications.

Conclusion: Anti-GBM antibody associated glomerulonephritis is rare with an estimated incidence of 1 patient per million population per year. This study is consistent with other reports of anti-GBM disease carrying poor renal prognosis. Patients surviving the acute presentation have a reasonable longer-term outcome.

II039/MP

The Impact of Diabetic Status on the Nutritional Profile of Patients on Stable Continuous Ambulatory Peritoneal Dialysis

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Aim: Malnutrition is associated with increased mortality in CAPD patients. It can be caused by a number of different factors. Diabetes has been reported to be a major influence on malnutrition. In long-term survivors however, other causes of malnutrition are likely to be more important influences. The aim was to determine whether the diabetic status in stable patients who survived the first year on continuous ambulatory peritoneal dialysis (CAPD) was associated with increased evidence of malnutrition

Methods: Thirty-one patients were studied over a period of 2 years. The following parameters were noted/assessed within 4 months of starting CAPD: age, sex, diabetic status, subjective global assessment (SGA), body-mass index, serum albumin, and dietary protein intake (DPI). Nineteen of 31 (61%) patients were diabetics. There were no significant differences in age, gender and ethnicity between diabetics and non-diabetics.

Results: When the whole group was analysed, 22/31 (71%) patients had SGA scores of 5 or less. The mean serum albumin was 30.7 g/L. Mean DPI was 0.9 g/kgm bodyweight/day. However, between diabetics and non-diabetics, there were no significant differences seen in the SGA scores, mean serum albumin, mean dietary protein intake or the BMI.

Conclusion: In patients who survived the first year of CAPD, although malnutrition was prevalent, diabetic status, however, did not seem to have influenced the nutritional profile. In these patients therefore it is possible that other factors may be contributing to malnutrition.

II040/MP

Vasomotor Reflexes are Dependent on Peak Flow and Volume of the Inspiratory Trigger

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Aim: A vasomotor reflex (VMR) is a physiological vasoconstrictive response triggered by a rapid and deep inspiratory gasp. This study investigates the relationship of the rate of inspiration and the inspiratory volume on the magnitude of the VMR in skin and muscle supplying vessels.

Methods: 12 healthy volunteers were tested for effect of lung volume and inspiratory flow on blood flow over the 4th digit tip and the ulnar artery using Doppler. Peak Inspiratory Flow (PIF) and Forced Inspiratory Vital Capacity (FIVC) were divided into 3 levels: low (PIF<1.0 l/min; FIVC<1.0 l), medium (PIF 1.0-1.5 l/min; FIVC1.0-1.5 l) and high level (PIF>1.5 l/min; FIVC>1.5 l). Each testing level was repeated thrice and repeated at 3 occasions (1 to 14 days) to determine test variability. VMR was defined as the pre and post inspiratory gasp difference in blood flow.

Results: The results for VMR at the 3 levels of PIF and FIVC are listed as VMR/PIF/FIVC. All the values are mean values. For the 4th digit: 10.4%/0.7/0.7; 33.0%/1.4/1.3; 58.5%/3.4/2.2. For the ulnar artery: 34.9%/0.8/0.7; 61.0%/1.5/1.3; 84.2%/3.3/2.1. Variability of VMR, for high level PIF and FIVC was small (p for all <0.05). Skin and muscle VMR significantly correlated with both PIF and FIVC. PIF showed slightly better correlation than FIVC (p for both <0.01).

Conclusion: VMRs show a significant direct correlation to both PIF and FIVC. This result needs to be considered when interpreting VMR as the effort of the triggering inspiratory gasp will affect the value of the VMR.

II041/MP

Stimulated Skin Wrinkling

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Aim: Compared to test of large nerve fibre function, there are few established tests of small nerve function. One established but technically demanding test of small nerve damage counts intraepidermal nerve fibre density (IENFD) obtained from skin biopsy. This study correlates 2 tests of small nerve function, water and EMLA® induced skin wrinkling, with IENFD.

Methods: Patients with sensory polyneuropathy had upper and lower limb neurophysiology. Water skin wrinkling involved immersion (40° C) for 30 min. EMLA® skin wrinkling necessitated covering distal 3rd, 4th and 5th digit tips with EMLA® cream for 30 min. Skin wrinkling was scored as 0 if the tip showed less than 2 wrinkling lines and as 1 if there was obvious wrinkling with 3 or more lines. Skin biopsy for IENFD was performed over the non-dominant hypothenar region. Immunohistochemical staining for nerve fibres used PGP 9.5 antibodies. IENFD was calculated from 3 sections and expressed as nerve fibers per millimeter.

Results: Skin biopsy and the 2 techniques of stimulated skin wrinkling were compared in 33 (13 male, 20 female; mean age 57 years, range 20-88) with predominant sensory neuropathy. Correlation of IENFD and wrinkling was significant for EMLA® (p = 0.046) but not for water (p = 0.546).

Conclusion: The small nerve fibre test of EMLA® induced skin wrinkling correlates with IENFD. The negative result for water immersion may be due to difficulty in interpretation of water results which are far less marked and clear than for EMLA®. EMLA® stimulated skin wrinkling may develop into a useful bedside small nerve fibre test.

II042/MP

Skin and Muscle Vasomotor Reflexes Follow by Sequential Inspirations in Healthy Adults

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Aim: This study investigates the relationship of the rate of inspiration and the inspiratory volume on the magnitude of the vasomotor reflexes (VMRs) in skin and muscle supplying vessels.

Methods: 12 healthy volunteers (between 21-65 years) were recruited. We tested the effect of varying lung volume and inspiratory flow on changes in blood flow over the skin of the 4th digit tip using Laser Doppler and over the ulnar artery using Ultrasound Doppler. In each subject, 9 sequential inspirations were tested. Peak Inspiratory Flow (PIF) and Forced Inspiratory Vital Capacity (FIVC) were divided into 3 levels: low (PIF<1.0 l/min; FIVC<1.0 l), medium (PIF 1.0-1.5 l/min; FIVC1.0-1.5 l) and high level (PIF>1.5 l/min; FIVC>1.5 l). Each testing level was repeated 3 times, and the average result taken. There was 1 minute interval between tests. Testing was repeated at 3 separate occasions. VMR was defined as the pre and post inspiratory gasp difference in blood flow and expressed as a percentage.

Results: The results for VMR at the 3 levels of PIF and FIVC are listed as VMR/PIF/FIVC. Mean for the 4th digit: 10.4%/0.7/0.7; 33.0%/1.4/1.3; 58.5%/3.4/2.2. For the ulnar artery: 34.9%/0.8/0.7; 61.0%/1.5/1.3; 84.2%/3.3/2.1. Variability of VMR, for high level PIF and FIVC, was small (p for all <0.05). Skin and muscle VMR significantly correlated with both PIF and FIVC. PIF showed slightly better correlation than FIVC (p for both <0.01).

Conclusion: VMRs show a significant direct correlation to both PIF and FIVC. This result needs to be considered when interpreting VMR as the effort of the triggering inspiratory gasp will affect the value of the VMR.

II043/MP

Aggressive Intervention Retards Progression of Nephropathy in Individuals With Type 2 Diabetes Mellitus

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Aim: To analyse the baseline and 6-month follow-up clinical data of patients with type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN).

Methods: We aimed to retard the progression of DN in T2DM patients by intensive patient education, metabolic and blood pressure control in a joint nephrologist-diabetologist diabetes nephropathy clinic (DNC). We studied the baseline profile of these patients and analysed the change of relevant metabolic parameters and renal function over 6 months with aggressive intervention.

Results: 53 subjects completed 6-months of follow-up. At baseline, these patients had moderately severe DN with 24-hour urine total protein and calculated creatinine clearance (using Cockcroft Gault equation with adjustment for gender) of 2.10 ± 2.59 g/day and 41.46 ± 30.74 ml/min respectively. After 6 months, HbA1c improved by 0.6 ± 2.0 % from a baseline HbA1c of 8.1 ± 2.3 % (p = 0.042) and there was a significant shift towards dual therapy with angiotensinogen converting enzyme inhibitors and angiotensin receptor blockers (p = 0.001). There was also a significant increase in number of antihypertensive agents used by 0.51 ± 1.07 (p = 0.001). We observed a non-significant trend towards improvement of systolic (baseline vs. 6-month follow-up, 154 ± 22 vs. 151 ± 25 mm Hg) and diastolic blood pressure (baseline vs. 6-month, 82 ± 11 vs. 79 ± 12 mmHg). As a cohort, the decline in calculated creatinine clearance over 6 months was minimal (0.70 ± 6.91 ml/min, p = 0.495).

Conclusion: The usual relentless progression of DN appeared to have been retarded in our cohort despite relatively modest improvement in known risk

factors. Continued risk factor modification and a longer follow-up will be necessary for further clarification of our preliminary data.

II044/MP

The Empirical Antibiotic Treatment of Continuous Ambulatory Peritoneal Dialysis (CAPD)-Related Peritonitis—Is It Appropriate?

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Aim: CAPD related peritonitis is serious and can be life-threatening if treatment is delayed. Empirical antibiotic at initial presentation is therefore necessary. This is affected by the likely results of the peritoneal fluid culture. To determine the appropriateness of current empirical antibiotic treatment in relation to peritoneal effluent culture result at presentation of CAPD-related peritonitis

Methods: All patients (n = 32) with peritonitis episodes in the NUH CAPD programme from 1/1/2002 till 31/12/2002 were studied retrospectively. There were 57 episodes of peritonitis. Microorganism culture, antibiotics used and the treatment outcomes were analysed. All patients received empirical intra-peritoneal vancomycin and gentamicin at presentation. Antibiotic treatment was subsequently altered according to culture and sensitivity results.

Results: Eleven of 57 episodes (19%) had negative cultures. Forty-one of 57 episodes (72%) were associated with 1 microorganism, 4/57 was associated with 2 microorganisms and 1/57 episodes was associated with 3 microorganisms. Of the 46 episodes with positive cultures, gram-positive organisms accounted for 40% and gram-negative organisms were responsible for 37%. The remaining were either fungus or negative culture. The 3 commonest gram-positive organisms isolated were coagulase-negative *Staphylococcus* (7 isolates), *Streptococcus* sp. (6 isolates) and *Staphylococcus aureus* (3 isolates). The 3 commonest gram-negative organisms isolated were *Klebsiella pneumoniae* (4 isolates), *E. coli* (3 isolates) and *Acinetobacter* sp (2 isolates). Response rate to this antibiotic regimen was 87%.

Conclusion: The current empirical intraperitoneal antibiotic regimen that covers both gram-positive and gram-negative organisms is appropriate in treating more than three-quarter of the CAPD-related peritonitis.

II045/MP

Clinical and Biochemical Profile of SAF Servicemen Referred to the Weight Management Clinic of a Tertiary Health Care Institution

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Aim: Obesity is increasingly being associated with many co-morbid conditions. Obesity as a public health problem has also been on the rise in Singapore. The present study is aimed at analysing the clinical and biochemical profile of 401 SAF servicemen referred for weight management in 2002.

Methods: A retrospective study was conducted on 401 consecutive SAF personnel referred for weight management. The clinical parameters analysed were age, body mass index (BMI), waist-hip ratio and BP. The biochemical parameters measured included fasting lipids and fasting glucose.

Results: The mean age of the study population was 32.16 years. All the patients were male. Lowest BMI was 26.3 and the highest was 53.1. The ethnic distribution was 74% Chinese, 17% Malays and 9% Indians. 26.1% of the study population were in the overweight category (BMI 25.0-29.9), 56% of patients had Class I obesity (BMI 30-34.9), 13.0% of patients had Class II obesity (BMI 35-39.9) and 4.9% had morbid or Class III obesity (BMI ≥ 40). Mean triglyceride levels in the increasing levels of obesity were 1.80, 1.97, 1.98 and 2.45 mmol/L, respectively. Mean fasting glucose levels were 5.04, 5.12, 5.82 and 6.23 mmol/L in the 4 categories of obesity, respectively. 14 % of the patients had elevated BP ($>140/90$), 42% had raised LDL

cholesterol levels and 15% hypercholesterolaemia.

Conclusion: Increasing degree of obesity in our local SAF servicemen is associated with increased risk of hyperglycaemia and hypertriglyceridaemia.

II046/MP

Predictive Factors of Outcome in Continuous Ambulatory Peritoneal Dialysis (CAPD)-Related Bacterial Peritonitis

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Aim: Bacterial peritonitis is a major determinant of patient morbidity and mortality. Awareness of factors that affect response to treatment is important in management. To study if a) age, sex, diabetic status, C-reactive protein (CRP) levels and peritoneal fluid cell counts and b) gram staining characteristic of the causative bacteria could predict the likelihood of successful resolution of the peritonitis.

Methods: Forty-four episodes (32 patients) of bacterial CAPD peritonitis episodes presented over 1 year were studied. Twenty-four occurred in male patients. The mean age was 64.8 ± 12.3 years. Sixteen of 44 episodes (36%) occurred in diabetics. Twenty-three of 44 episodes (52%) were due to gram-positive organisms. All episodes were initially treated with empirical intraperitoneal vancomycin and gentamicin. The mean serum albumin and C-reactive protein (CRP) were 27.7 ± 7.1 g/l and 19.6 ± 18.3 mg/dl, respectively. The mean peritoneal fluid cell count was 4815 ± 5969 per microlitre. Forty-one of 44 episodes (93.2%) resolved after antibiotic treatment (responder group)

Results: There was no significant difference between the mean ages, diabetic status, initial gram stain results, gender, serum albumin concentrations and peritoneal fluid cell counts between the responders and non-responders. The mean CRP of the responder subgroup was significantly lower compared to the non-responders (14.9 ± 10.4 vs 49.3 ± 31.4 mg/dl), ($p < 0.005$).

Conclusion: Only lower CRP was associated with response to antibiotic treatment. Diabetic status, age, gender, initial gram stain results, peritoneal fluid cell count and serum albumin were not associated with treatment outcome.

II047/MP

Treatment Outcome of Continuous Ambulatory Peritoneal Dialysis (CAPD)-Related Peritonitis at National University Hospital (NUH)

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Aim: Management of CAPD-related peritonitis involves consideration of appropriate use of antibiotics and timely removal of Tenckhoff catheter if treatment fails. To review treatment outcome of CAPD-related peritonitis in relation to culture results.

Methods: Forty-five patients, with CAPD-related peritonitis presented from 1/1/2002 till 31/12/2002, were included. Data on microorganism culture and antibiotics used were collected and analysed in relation to treatment outcome. Treatment failure defined as failure of significant clinical improvement after initiating therapy for 72 hours that required Tenckhoff catheter removal.

Results: There were 55 episodes of CAPD-related peritonitis between 1/1/2002 and 31/12/2002. Forty-four episodes were due to bacteria, while 11 episodes were culture negative. All patients received intra-peritoneal vancomycin and gentamicin at presentation. Twelve episodes required the use of either ciprofloxacin or ceftazidime. Fifty episodes (90.9%) resolved after treatment while 5 episodes (9.1%) had treatment failure. For treatment failure group, culture results revealed *Pseudomonas aeruginosa* (2), *Staphylococcus aureus* (1) and culture negative (2). Percentage of treatment failure by different culture results were 6.8 and 18.2 for bacterial and culture negative peritonitis, respectively. Interestingly, about one-fifth of culture negative peritonitis group required Tenckhoff catheter removal. There may be an increase trend of catheter removal in the culture negative group, however, this has to be further elucidated.

Conclusion: Our empirical antibiotic regimen has a very high success rate for treatment of CAPD peritonitis. However, effective treatment has to be balanced with culture and sensitivity results and prudent use of antibiotics, which needs to be tailored accordingly.

II048/MP

A Comparison of Different Clinical Prediction Rules for Neuroimaging in the Evaluation of Dementia

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Aim: There is ongoing controversy regarding the need for CT imaging in the evaluation of dementia. This study aims to compare different prediction rules that have been proposed in a single, common cohort of patients presenting to a memory clinic for the evaluation of possible or worsening dementia. 4 clinical prediction rules were compared.

Methods: A retrospective study was done on 210 patients attending the TTSH memory clinic. All these patients had at least 1 CT brain done and all CT scans were reported by a single radiologist. These patients were also assessed using the 4 clinical prediction rules, which includes Bradshaw et al, Dietch, Larson [high risk] and the Canadian Consensus Conference on the Assessment of Dementia [CCCAD].

Results: Out of the 210 patients, 11 [5.2%] had surgically treatable conditions. The mean age was 73.9 [SD 8.7]. 79 [37.6%] were males and 131 [62.4 %] were females. The clinical diagnosis was Alzheimers dementia in 38.6%, vascular dementia in 29% and mixed in 2.4%. Clinical predictive rule Sensitivity [95% CI] % Specificity [95% CI] % Bradshaw et al 27.3 [6.0-61.0] 75.4 [68.8-81.2] Dietch 54.5 [23.4-83.3] 54.3 [23.4-83.3] Larson et al [high risk] 70.0 [34.8-93.3] 62.2 [34.8-93.3] CCCAD 72.7 [39.0-94.0] 21.1 [39.0-94.0] *p*-value from Fisher's exact test *CI* calculated from exact binomial distribution.

Conclusion: Clinical prediction rules have limited sensitivity and specificity. Further studies are needed to evaluate the usefulness of intervention in patients with space occupying lesions and also the cost effectiveness of CT neuroimaging in the evaluation of dementia.

II049/MP

Crescentic Glomerulonephritis in Association with Anti-neutrophil Cytoplasmic Antibody (ANCA): A Report of the Clinic-Pathological Experience over 5 Years in a Single Centre

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Aim: To study the clinico-pathological features of patients with ANCA-associated crescentic glomerulonephritis seen in the National University Hospital from 1 September 1997 to 31 May 2003.

Methods: Four hundred twenty-seven adequate renal biopsies from 427 patients were reviewed. Ten of the 427 (2%) biopsies showed crescentic glomerulonephritis. Of these, 3 (30%) were associated with positive ANCA and the associated clinical histories were retrospectively studied. Biopsies with associated antglomerular basement membrane antibody, lupus nephropathy and IgA nephropathy were excluded.

Results: The ages of the 3 patients were 23, 64 and 67 years, respectively. Their serum creatinine levels at presentation were 66, 176 and 144 umol/litre respectively. All presented with lethargy, anorexia, weight loss and epigastric pain (and vomiting). All had microscopic haematuria and proteinuria. One was also febrile and 1 other had hemoptysis. All developed rising creatinine levels during their clinical course. All had 50% or more cellular/fibrocellular crescents in their renal biopsies. None had necrotizing vasculitis. All received corticosteroids and 2 were additionally treated with intravenous cyclophosphamide. One patient had pulmonary haemorrhage, required plasma exchange, ventilation and dialysis, and died of gram-negative sepsis after 5 months in hospital. The 2 other patients were followed up for 1 month

(subsequently defaulted follow-up) and 9 months. At this time the serum creatinine levels were 173 and 248 umol/litre respectively.

Conclusion: ANCA-associated crescentic glomerulonephritis is a rare condition in our experience. However, early diagnosis and immunosuppressive therapy can lead to stable renal function.

II050/MP

Clinical Profile and Electrophysiological Study of Patients with Traumatic Brachial Plexus Injury

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Aim: To study the clinical, electrodiagnostic and magnetic resonance imaging (MRI) features of patients with traumatic Brachial Plexus Injury (BPI) seen at the electrophysiology laboratory of a tertiary urban hospital in Singapore over a 2-year period.

Methods: The case-records of 24 patients were reviewed.

Results: Ten patients sustained BPI in motorcycle accidents. In 9, the site of injury was at the trunks and/or roots and recovery was poor. Three had root avulsion and 2 cervical cord contusion evident on MRI. Four were involved in car accidents. No single mechanism of injury was discernible. Most commonly only a single trunk was injured and recovery was good. Two patients had MRI, which were unremarkable. Eight patients had BPI as result of a fall on out-stretched arm or being struck on the shoulder/head. The injury was confined to single trunk and/or nerve (most commonly axillary nerve) and at least partial recovery was observed. One patient sustained injury to the lower cervical roots and the long thoracic nerve after carrying heavy loads. One patient sustained injury to the middle and lower trunks after a coronary angiogram. Subsequent recovery was partial.

Conclusion: Motorcycle accidents appear to be the most common cause of BPI in Singapore. The patients sustain either root avulsion or axonal injury and recovery is poor. BPI from car accidents and falls are often partial and better recovery is expected.

II051/MP

Validation of a Modified WHO Protocol for Stroke Screening

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Aim: This sub-study was performed to validate a modified version of the WHO Protocol for Epidemiologic Studies of Neurologic Disorders as a screening tool for stroke in Singapore.

Methods: The main study is a community-based prevalence survey of neurological disorders among Singaporeans aged > 50 years. Study subjects undergo a face-to-face interview by trained interviewers using a modified version of the WHO Protocol. Phase I "failures" are reviewed in Phase II by consultant physicians to rule in or out the diagnosis of stroke. The sub-study sample size of 1300 was derived using an estimated sensitivity of 0.9, specificity 0.8 and precision 0.03. As 83% of the main study subjects passed Phase I, 83% of 1300 (=1079) subjects were randomly selected from among "Phase I passes" and invited to a clinical review by trained physicians to rule in or out a diagnosis of stroke. The stroke status of the remaining 17% (= 218) was derived from the results of their Phase II review. Data were analysed using SPSS programme.

Results: Of the 1079 "Phase I passes", 40 could not be contacted. Of the remaining 1039, 17 were diagnosed to have stroke. Of the 218 "Phase I failures", 46 were diagnosed to have stroke. This resulted in a sensitivity of 0.73 (95%CI 0.71-0.75), specificity of 0.86 (95%CI 0.84-0.88), positive predictive value (PPV) of 0.21 (95%CI 0.19-0.23), negative predictive value of 0.98 (95%CI 0.97-0.99).

Conclusion: The modified WHO protocol has a fairly sensitivity and specificity for screening for stroke, and is a suitable screening tool for stroke in Singapore. However, it has a low PPV due to low prevalence of disease.

II052/MP

Management of Status Epilepticus in Patients Who Develop Hypersensitivity to Phenytoin and Phenobarbitone

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Aim: Patients with status epilepticus (SE) treated with phenytoin and phenobarbitone may develop anticonvulsant hypersensitivity syndrome. The purpose of our study was to evaluate the efficacy of continuous high dose intravenous lorazepam with concurrent topiramate or gabapentin in this condition.

Methods: All patients with SE who developed phenytoin and phenobarbitone hypersensitivity were identified. Continuous intravenous lorazepam infusion was commenced at a rate of 1 mg/hr and titrated upwards at increments of 1 mg/hr while monitoring for clinical and electrographic seizures. Once seizures were aborted the infusion rate was continued for 24 hours and then titrated downwards at a rate of 1 mg/hr/day. If seizures recurred the infusion rate was increased accordingly. Concurrent with lorazepam, either topiramate or gabapentin was started and titrated to therapeutic effect.

Results: Three patients were identified with a median age of 34 (range 15-61) years. Hypersensitivity syndrome occurred within 13-19 (median 14) days of commencement of medication. Intravenous lorazepam aborted seizures in all cases. The median duration of lorazepam therapy was 26 days (range 21-28) and the maximal dose of lorazepam per day ranged from 12 to 96 mg. Fluids and vasopressors were required for all patients. Topiramate was used as add-on therapy in 2 cases (daily doses of 150 mg and 400 mg) and gabapentin in 1 case (daily dose 1800 mg). Outcome was death in 1 patient and mild disability in 2 patients.

Conclusion: Continuous high dose intravenous lorazepam, together with concurrent topiramate or gabapentin, was successful in abolition of seizures in anticonvulsant hypersensitivity syndrome.

II053/MP

CT Perfusion in Brain Tumours

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Aim: Dynamic contrast-enhanced CT perfusion imaging may be useful in assessing tumour angiogenesis. We analysed the permeability surface (PS) constant values in intracranial tumours of different histologic types, to determine if there are differences in the microvascular permeability.

Methods: Fourteen patients with newly diagnosed intracranial tumours underwent CT perfusion using a helical CT scanner. The CT perfusion study was obtained on a single section at the largest tumour diameter identified on non-contrast CT scan. Images were acquired during dynamic intravenous injection of 50 ml of non-ionic iodinated contrast medium (300 mg/I) at 4ml per second. The permeability surface (PS in ml/100g/min) constant, cerebral blood volume (CBV) and flow (CBF) were analysed using Functool post-processing software.

Results: Calculations in 2 of 15 studies failed due to inability to identify an artery or vein in patients with high convexity meningiomas. In the other 12 pre-treatment studies, elevation of PS, CBV and CBF was noted compared to normal brain parenchyma. One patient with metastases who underwent repeat study after radiotherapy demonstrated a small decrease in PS constant together with marked decrease in vasogenic oedema and tumour size.

Conclusion: Despite current limitations due to single-section coverage of anatomy, CT perfusion may be useful in the non-invasive assessment of brain tumour biology and response to therapy.

II054/MP

Cerebrospinal Fluid Orexin in Aneurysmal SAH

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Aim: To investigate the relationship of the orexinergic system to the clinical severity of aneurysmal SAH.

Methods: CSF levels of orexin-A from 15 patients with diffuse spontaneous SAH were measured using a commercially available EIA kit.

Results: Orexin levels in the CSF of patients with aneurysmal SAH are reduced and that undetectable levels of orexin in CSF samples obtained within the first day following the ictus correlates significantly with low GCS (Glasgow Coma Scale). The converse situation, where detectable levels of orexin in these same samples exhibit a relationship to higher GCS.

Conclusion: Hypothalamic damage in SAH may lead to injury of the hypothalamic orexinergic neurons and thus lead to lowered orexin levels. Orexin levels could be established in future study as a robust prognostic indicator for survival and outcome after aneurysmal SAH.

II055/MP

Fluorescence In-situ Hybridization (FISH) – Is It a Sensitive Tool to Detect Low-grade Mosaicism in Patients With Turner Features but a Normal Karyotype?

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Aim: Turner syndrome is a sex chromosome disorder characterised by short stature, gonadal dysgenesis and somatic stigmata. The most common karyotype in an individual with Turner syndrome is 45,X. 45,X/46,XX or 45,X/46,XY mosaics account for 15% to 30% of cases. However, many mosaic patients with Turner features are reported to have a normal karyotype on routine cytogenetic analysis showing a phenotype/genotype discrepancy. The aim of this study was to use the more sensitive FISH technique to detect whether the suspected 45,X cell line can be detected in the peripheral blood.

Methods: Mitogen-stimulated short-term peripheral lymphocyte cultures were set up and cytogenetic analysis with trypsin-leishman banding using standard techniques was performed on 11 cases and compared with control samples. 100 G-banded metaphase spreads were analysed in each case. FISH analysis using X centromeric probes (DXZ1) and Xterminal probes was done and about 5000 cells (both metaphases and interphases) were observed. A statistical analysis of the samples has been done to detect whether there is a significant difference.

Results: A low percentage of the abnormal monosomic cell line was observed in all the patient samples with the more sensitive FISH technique, which could not be detected with the routine cytogenetic methods.

Conclusion: FISH proved useful and sensitive in the detection of the mosaic monosomic cell lines, which could not be detected by traditional cytogenetic G-banding techniques. A larger number of samples needs to be analysed and an appropriate cut-off level needs to be determined before this test can be offered to patients.

II056/MP

Treatment of Macular Oedema in Central Retinal Vein Occlusion Using Deep Posterior Subtenon Injection of Corticosteroids

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Aim: Macular oedema forms the main cause of permanent loss in vision in patients with central retinal vein occlusion (CRVO). Our study aims to evaluate the effect of deep posterior subtenon injection of corticosteroids on macular oedema in CRVO.

Methods: It is a non-randomised study. Recruitment criteria included patients with ischaemic CRVO with persistent macular oedema of more than 3 months following panretinal and macular grid pattern photocoagulation treatment. Deep posterior subtenon triamcinolone 40 mg was injected via the infero-temporal conjunctival fornix. Outcome measures included post-treatment fundal fluorescein angiogram (FFA) and visual acuity at the 6th week.

Results: A total of 6 eyes were recruited into the study. All 6 eyes underwent panretinal and macular grid pattern photocoagulation treatment. Pretreatment FFA showed gross macular oedema and leakage in all 6 eyes. Pre-treatment visual acuity was counting fingers closely in all 6 eyes. Following deep posterior subtenon triamcinolone injection, post-treatment FFA at 6th week showed significant reduction in macular oedema and leakage in all 6 eyes. Visual acuity improved to 6/24 in 2 eyes, 6/60 in 2 eyes on the Snellen acuity chart at the 6th week post-treatment whereas the remaining 2 eyes showed modest improvement from counting finger to 6/120 due to macular ischaemia. No complications nor rise in intraocular pressure was noted in all 6 eyes.

Conclusion: The results of the study suggest that deep posterior subtenon injection of steroid therapy may be used as a safe treatment modality for persistent macular oedema in CRVO, resulting in reduction of macular oedema. Improvement in visual acuity may be achieved in cases not compromised by macular ischaemia.

II057/MP

Optociliary Shunt Vessels in Diabetics— A Case Series

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Aim: Optociliary shunt vessel is classically described to be associated with optic nerve sheath meningioma with the triad symptoms of optociliary veins, disc pallor and visual loss. Other clinical settings include retinal vein occlusion, chronic glaucoma, meningioma and rarely as a congenital form. We report an interesting case series of 3 patients with diabetes mellitus presenting with optociliary shunts.

Methods: Patients who underwent diabetic retinopathy screening were referred to the eye clinic for abnormal findings. A total of 3 patients with optociliary shunt vessels were noted between year 2000 and 2001. The appearance of an optociliary vein was documented with fundus photography and fundal fluorescein angiography.

Results: All 3 patients had bilateral mild non-proliferative diabetic retinopathy with 1 having bilateral diabetic maculopathy in addition. Fluorescein angiography showed classical features of acquired optociliary shunts with no leakages. Systemic review did not show any secondary cause of the optociliary shunts.

Conclusion: Our series showed that optociliary veins can be associated with diabetes mellitus. The authors postulate that it may be due to venous insufficiency secondary to the process of diabetic microangiopathy and venous stasis.

II058/MP

Diabetic Retinopathy Screening: Knowledge, Confidence, Perceptions and Practices of Singaporean Optometry Students

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Aim: To determine the knowledge on diabetic retinopathy, confidence in screening, and perceptions and practices regarding diabetic retinopathy screening and patient education among Singapore optometry students.

Methods: Self-administered questionnaire survey was conducted on all final-year optometry students in Singapore.

Results: The entire class of 36 students responded to the survey. The students performed well in a test on knowledge regarding diabetic retinopathy with a mean score of 9 out of 10 questions. They scored a mean of 5.5 on a confidence level scale of 0 to 10 for diabetic retinopathy screening. Seventy percent of them would screen their patients for diabetic retinopathy and forty-four percent would refer them to the ophthalmologists. They perceived that the responsibility in screening rests with the optician (0%), optometrist (97%), physician treating the patient's diabetes (86%), ophthalmologist treating the patient for an eye condition unrelated to diabetes (92%), and non-ophthalmology medical specialists treating the patient for a diabetic complication (28%). They also perceived that the responsibility in patient education rests with the optician (28%), optometrist (100%), physician

(100%), ophthalmologist (89%), and non-ophthalmology medical specialists (50%).

Conclusion: Optometry students have a good knowledge in diabetic retinopathy. Although the confidence level in screening was not high, the majority of them felt that they were responsible in screening and educating patients on diabetic retinopathy.

II059/MP

Idiopathic Submacular Haemorrhage

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Aim: The majority of submacular haemorrhages are secondary to angiographically detectable lesions such as choroidal neovascularisation and idiopathic polypoidal choroidal vasculopathy. Idiopathic cases are rare. We report 2 cases to highlight their management and clinical outcome.

Methods: Two case reports.

Results: Two men aged 53 and 66 years presented with a sudden onset of painless loss of vision in 1 eye. Their visual acuity in the affected eye was counting fingers. Fundal examination disclosed thick submacular haemorrhage in the affected eyes. Both patients received intravitreal injections of tissue plasminogen activator (tPA) 25 ug in 0.1 ml and 0.3 ml 100% perfluoropropane (C₃F₈) in the affected eye within 24 hours of presentation. Both patients were instructed to position their heads facing downwards for 1 week. Post-treatment fluorescein and indocyanine green angiography were performed after adequate blood displacement from under the fovea in the patients, respectively. The patients' vision improved to 6/12 and 6/24 at 1 month post-treatment. Displacement of blood from the fovea was almost complete after 1 week of prone positioning in both patients. Post-treatment fluorescein and ICG angiography did not disclose any vascular lesions which could account for the submacular haemorrhage in both patients. There was no intra- or post-operative complication and the vision remained stable in both patients at 6 months post treatment.

Conclusion: The outcome of idiopathic submacular haemorrhage following intravitreal tPA and gas injections and post-operative prone positioning is good.

II060/MP

Malignant Germ Cell Tumors in a Paediatric Population in Singapore – National University Hospital Experience

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Aim: Malignant germ cell tumours (MGCT) represent 3% of childhood tumours. A higher percent may be present in Asian population. This is our single institution experience in young children with this rare tumour, its characteristics and the outcomes.

Methods: A retrospective chart review of all paediatric oncology patients seen at the centre between November 1997 and April 2003 revealed 13 patients with MGCT. Median age was 8.8 (range 1.2-15.2 years) and 69% were male (n = 9). Primary sites were gonadal (n = 6), intracranial (n = 5), and mediastinal (n = 2). Histology revealed 5 seminomatous and 8 non-seminomatous. There was 1 stage IV while the rest were stages I-II. Therapy for these patients consisted of cisplatin-based chemotherapy plus surgery for 6 patients, surgery alone in 3, and combined therapy (Surgery ± Chemo ± Radiation) in 4.

Results: Median time of survival after diagnosis of the MGCT was 1.2 (range 0.2- 3.1 years). At the time of analysis, 8 patients were alive and free of disease. One patient with intracranial primary relapsed after 6 months and was alive with disease at 39 months after primary diagnosis. One patient with pineal teratoma, treated with surgery, chemotherapy and radiation, developed leptomeningeal disease and died at 21 months after initial diagnosis.

Conclusion: The outcome of our patients, including the extra-gonadal primary, was good; although the sample size was limited. Surgery alone or

multimodal therapy is essential in these patients for improved prognosis. Further studies including large prospective trials and new treatment approaches are needed for improved survival.

II061/MP

Computer Aided Paediatric Prescribing

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Aim: To compare prescription errors by traditional and computer aided prescribing.

Methods: Computer-based (not handwritten) prescriptions are the norm in our institution. Algorithms for 2 common drug dosages (paracetamol and promethazine), based on patient weight, were developed by paediatricians and incorporated into the computer programme. Doctors could choose to prescribe their own dose (traditional) or enable the computer to suggest the dose (computer aided). This suggested dose could be overridden but an alert was given if it was outside the recommended dose range.

Results: Of 2042 paracetamol and promethazine prescriptions, 57% utilised computer aided prescribing. The percentages within each doctor group were: medical officer 88%, house officer 48%, registrar 46%, associate consultant 38% and consultant 29%. Overall error rate (under-dosing, overdosing or dose omission) of computer aided prescriptions was 11.1% compared to 26.4% for traditional prescriptions [relative risk (R) 0.42, 95% confidence intervals (CI) 0.35-0.51, χ^2 $p < 0.001$]. Most errors were underdosing – 9.9% of computer aided, 20.7% of traditional prescriptions (R = 0.48, CI = 0.39-0.60, χ^2 $p < 0.001$). Least errors were overdosing – 0.9% of computer aided, 3.0% of traditional prescriptions (R = 0.29, CI = 0.14-0.59, χ^2 $p < 0.001$). Error rates for computer aided versus traditional (brackets) prescriptions by doctor grade were: medical officer 3.4% (29.4%), registrar 8.1% (22.5%), associate consultant 19.5% (30.9%), consultant 26.7% (22.8%) and house officer 28.4% (32.3%). House officer, associate consultant and consultant changes were not significant.

Conclusion: Computer aided prescribing halved errors and was particularly effective in reducing overdoses and dose omissions. Middle grade staff took more advantage of these benefits.

II062/MP

Home Assisted Ventilation for Children in Singapore: A New Ancillary Service for the 21st Century

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Aim: To evaluate the outcome of children on Home Assisted Ventilation (HAV) under our care.

Methods: A total of 16 children on HAV from 1997 to April 2003 (2 severe obstructive sleep apnoea, 6 neuromuscular disease [NMD], 3 central hypoventilation [CH] and 5 chronic lung disease [CLD]) were monitored for improvement in gas exchange, complications and survival.

Results: pCO_2 improved in the latter 3 subgroups after initiation of HAV, best seen in the NMD subgroup (mean pCO_2 decreased from 57.45 to 45.25 mmHg, $p = 0.08$). Oxygenation, impaired only in the CLD group, also improved (mean paO_2 increased from 74.50 to 92.33 mmHg, $p = 0.44$). The median duration of HAV was 9.0 months (range 1 to 60 months). Apart from 3 deaths (1 each from CLD, CH and NMD subgroups), all patients are alive and well. One patient with an indeterminate form of NMD was weaned off HAV. There were minimal complications from HAV.

Conclusion: Overall, improvement in gas exchange was observed but did not reach statistical significance due to our small cohort. Developed countries that have established domiciliary ventilation programmes in place report good outcomes and quality of life (QOL). At present, our patients and their families are coping well and assessment of QOL is underway. Domiciliary ventilation as an ancillary service needs to be further evaluated and developed in Singapore

as it will help to reduce the length of hospitalisation for patients with chronic respiratory insufficiency; they can be discharged once the acute problems have been treated and the care of chronic problems continued at home.

II063/MP

Characteristics of Cancer Patients Referred to a Palliative Medicine Service in a Tertiary Acute Care Hospital in Singapore

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Aim: The department of Palliative Medicine at the National Cancer Centre provides a consultative service to inpatients at the nearby Singapore Hospital. Although a diagnosis of cancer is not a prerequisite, the majority of patients have advanced malignancy. A retrospective study was undertaken to determine the characteristics of cancer patients referred for palliative care, and to see if there were any differences between those patients referred by Medical Oncology compared to other specialties.

Methods: Inpatient referrals over a 1-year period were analysed to determine the demographic and clinical characteristics at the point of referral. Data on prognosis and place of death were also collected.

Results: 98 patients were referred by Medical Oncology and 245 by other specialties, mainly surgical. There were no significant differences between the 2 groups in terms of gender, performance status at referral, prognosis or place of death. However, Medical Oncology patients tended to be younger and were more likely to have received anti-cancer therapy. They also reported more symptoms and were more likely to have pain (77% vs 64%) as well as psychological and care concerns.

Conclusion: In general, patients referred to the Palliative Medicine service had poor functional status, short prognosis and multiple symptoms. Patients referred from Medical Oncology frequently had pain, and had more symptoms than patients from other specialties. There appears to be a trend towards more referrals from surgical specialties, which is seen as a positive development as it reflects increasing awareness and acceptance of Palliative Care within the acute hospital setting.

II064/MP

Clinical Utility of Urine Myoglobin Measurement

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Aim: Urine myoglobin measurement is presently not performed onsite. Samples are instead sent to another laboratory for testing. This study was designed to assess the clinical utility of urine myoglobin measurement via a retrospective audit of urine myoglobin requests, and describe the demographic details, laboratory findings and final diagnoses of patients suspected of myoglobinuria.

Methods: A retrospective audit was performed urine myoglobin requests received from September 2000 to August 2001. Demographic details (age, sex) and laboratory results (peak CK, aldolase, creatinine) from the laboratory information system and final discharge diagnoses from the electronic discharge summary were extracted.

Results: 115 samples were received, with 85 samples (79 patients) having discharge summaries available. Mortality rate was 20% [positive predictive factors on multivariate analysis were peak creatinine ($p < 0.01$) and discharge diagnosis of sepsis ($p < 0.05$) and negative predictive factor was discharge diagnosis of fitting ($p = 0.08$)]. Commonest discharge diagnoses were: infection 39%, rhabdomyolysis 16%, fitting 13%, myositis 8% and NMS 5%. Aldolase was the best independent clinical or lab parameter which differentiated final diagnosis of rhabdomyolysis from others ($p < 0.01$). No clinical or lab parameter separated rhabdomyolysis from myositis diagnoses. 48% of urine myoglobin results were available to clinicians only after patient discharge. No urine myoglobin results were positive in the year of the audit, or in the subsequent 2.5 years.

Conclusion: Urine myoglobin measurement is not of value in assessing patients suspected of myoglobinuria and such requests should be declined.

This finding agrees with published recommendations (Ann Clin Biochem 2000; 37:581-7).

II065/MP

Changes in Haematological Parameters in Severe Acute Respiratory Syndrome

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Aim: Severe acute respiratory syndrome (SARS) is an emerging infectious disease. Mortality amongst patients requiring intensive care (ICU) management is significant. We studied changes in haematological parameters according to the day of symptom onset in 185 probable SARS patients aiming to identify early markers helpful in excluding SARS and identifying patients with poorer prognosis.

Methods: Probable SARS patients identified from 15 March to 12 May 2003 were included in the study. Patients were divided into ICU and non-ICU groups. Median values of hematological parameters were analysed according to the day of symptom onset. Univariate and multivariate analyses were carried out to identify prognostic factors for patients needing ICU admission.

Results: White cell (WCC), absolute neutrophil (ANC), absolute lymphocyte (ALC) and platelet counts followed a v-shaped trend with the nadir at day 6 or 7 after symptom onset. An exception is ALC for the ICU group which had not reached the nadir by day 12. None of our patients had a platelet count $<80 \times 10^9/L$ and WCC $<2 \times 10^9/L$ in the first 5 days of symptoms which is when most patients present. On multivariate analysis, only age is an independent predictor for ICU admission (odds ratio per decade of life, 1.68; 95% confidence interval 1.32 to 2.14; $p < 0.001$).

Conclusion: WCC and platelet thresholds may allow early clinical exclusion of patients suspected of SARS. Although patients needing ICU admission tend to have persistent lymphopenia and higher WCC and ANC, these are not independent predictors for ICU admission.

II066/MP

Questionnaire Survey on the Main Causes of Hypoglycaemia in Diabetic Patients in a Teaching Hospital

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Aim: To determine the main causes of hypoglycaemia in the National University Hospital, Singapore and to formulate recommendations to prevent the occurrence of hypoglycaemia in our patients.

Methods: The study included diabetic adults who were admitted to the National University Hospital for hypoglycaemia with symptoms (neuroglycopenic and/or adrenergic) and a biochemical confirmation of a capillary glucose level of 3 mmol/L or less. The data were collected over a period of 4 months (September 2002 to January 2003) and were obtained from patients' casenotes during hospitalisation.

Results: Of the 74 patients included in this study, 64.9% were over 65 years, 71.6% were female and 87.8% were type 2 diabetics. The mean plasma glucose level was 2.02 ± 0.49 mmol/L and the mean HbA1c was found to be $7.47 \pm 1.85\%$. With regard to treatment, 31.1% of patients were being treated with insulin alone, while 21.6% were being treated with a sulphonylurea in combination with metformin. The major causes of hypoglycaemia in this study were found to be poor oral intake (55.4%), defined as either missed meals or a decreased intake of food and/or antidiabetic drug therapy (25.7%). From this study, the following recommendations were made: (1) Education of patients and caregivers on the need for regular meals, (2) Adherence to prescribed diabetic therapy, including the proper action to take when unwell and (3) Education of patients about home glucose monitoring.

Conclusion: Poor oral intake and drug therapy were the major causes of admissions for hypoglycaemia in this study. In order to prevent the occurrence of hypoglycaemia, recommendations were formulated to address these major causes.

II067/MP

A Retrospective Pilot Study of Suspected Beta-Lactam Allergy in Patients Prescribed Vancomycin in a General Hospital

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Aim: To examine the prevalence of beta-lactam allergy in patients administered vancomycin.

Methods: A random sample of 294 inpatients who had vancomycin prescribed between 1 January 2001 and 31 December 2001 were analysed with regards to indication for vancomycin use, history and type of suspected penicillin or cephalosporin allergy and diagnostic probability of beta-lactam allergy (determined by an allergy trained investigator [BYHT] using retrospective chart review and recorded opinion of the allergist if there was a referral).

Results: Forty-one (13.9%) patients with suspected beta-lactam allergy were given vancomycin on 43 separate occasions. The most common reasons for vancomycin use were pre-operative prophylaxis (16 episodes, 37.2%) and treatment of methicillin resistant *Staphylococcus aureus* infections (12 episodes, 27.9%). Retrospective chart review revealed 12 cases suggestive of probable beta-lactam allergy, 8 cases not suggestive and 21 indeterminate due to incomplete documentation. Only 3 of the 12 probable cases were suggestive of immediate hypersensitivity. Among 11 patients who were referred to an allergist prior to vancomycin administration, beta-lactam allergy was confirmed in 7 cases (63.6%) of whom only 2 (28.6%) were of immediate-type hypersensitivity, thus limiting the role for skin testing and penicillin desensitisation.

Conclusion: The prevalence of true beta-lactam allergy among patients prescribed vancomycin is low. Suspected beta-lactam allergy was not the reason why the majority of patients received vancomycin. Evaluation by an allergist may be useful in accurately diagnosing true beta-lactam allergies. This may reduce unnecessary vancomycin use, adverse reactions and development of vancomycin-resistant infections.

II068/MP

Predicting Psychosis From the Initial Prodrome – Chasing the Myth, a Local Perspective

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Aim: The prodrome of a psychotic disorder like schizophrenia is an elusive period with varied symptomatology, and is a critical time in the neurodevelopmental pathophysiology of psychosis with opportunities for early diagnosis and intervention once it can be reliably identified and studied. We therefore aimed to identify prodromal symptoms predictive of psychosis from 2 independent groups of young adult first-episode psychosis patients.

Methods: Firstly, semi-structured interviews of 30 patients with first-episode psychosis and 34 with non-psychotic disorders were used to retrospectively describe prodromal psychopathology (by THY and AYG), which were further explored using principal components analysis, logistic regression and the receiver operating characteristic (ROC) curves to narrow down prodromal symptoms that were sensitive and specific. Secondly, the sensitivity of these symptoms was tested against prodromal symptoms similarly elicited (by YJT) from an independent group of 50 first-episode psychosis patients.

Results: Principal components analysis yielded 1 component loaded with cognitive symptoms, attenuated negative symptoms, functional deterioration and perceptual disturbances, and another component loaded with neurotic symptoms. Only the first principal component was predictive of subsequent psychosis (OR 20, 95%CI 5.2 to 76, $p < 0.001$). From ROC analyses, having 3 or more of 15 cognitive, functional deterioration, attenuated negative or perceptual symptoms predicted psychosis with an approximate sensitivity and specificity of 0.8. This criterion identified patients from the second group with a sensitivity of 1.0.

Conclusion: Cognitive, functional deterioration, attenuated negative and

perceptual symptoms may be sensitive indicators of prodromal phenomena in our population. Applications may exist in the prospective screening and study of high-risk patients.

II069/MP

The Enigma of Urinary Retention in Post-Traumatic Minimally Responsive State: Single Case Report

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Aim: We report an unusual case of a 43-year-old female with post-traumatic minimally responsive state and tetraplegia who developed acute urinary retention 4 months into her inpatient rehabilitation programme after traumatic brain injury. Post-void residual urine volumes were 400 mL and urodynamic studies confirmed the presence of detrusor acontractility. Subsequent management involved 4-hourly intermittent catheterisation.

Methods: Concomitant spinal cord injury, faecal impaction, diabetes mellitus and bladder paralytic drugs were absent. Possible aetiologies for her bladder paralysis included treatments instituted for severe spasticity involving all 4 limbs: oral Dantrolene Sodium 300 mg/day, intramuscular Botulinum toxin A (Dysport 500U) to bilateral upper limbs and partial alcohol neurolysis of the right sciatic nerve for severe knee flexor spasticity. Oral or intrathecal Baclofen was not used in this patient.

Results: Spontaneous voiding resumed with reduction of Dantrolene Sodium to 150 mg/day with eventual attainment of urinary catheter-free status within 1 week and urodynamic studies documented return of detrusor activity.

Conclusion: This is the first reported clinical case of urinary retention following spasticity management involving therapeutic doses of Dantrolene Sodium. Patients in minimally responsive states after traumatic brain injury may be at increased risk of urinary retention due to profound immobility, motor weakness, aphasia, unawareness and brainstem/pontine injury. Those who receive high-dose Dantrolene Sodium therapy for treatment of spasticity may benefit from routine monitoring of voiding status and post-void residual urine volumes to detect urinary retention.

II070/MP

Molecular Genetic Analysis of a Patient with Late Onset Multiple acyl-CoA-Dehydrogenase Deficiency (MADD)

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Aim: This study aims to characterise the molecular genetic status of a patient with late onset multiple acyl-CoA-dehydrogenase deficiency (MADD). MADD is metabolic disorder associated with glutaric aciduria caused by deficiency of either electron transfer flavoprotein (ETF) or electron transfer flavoprotein ubiquinone oxidoreductase (ETF-QO).

Methods: We conducted genetic analysis on the patient with clinical features and biochemical data suggesting late onset MADD. Mutational analysis focusing on the 3 genes (ETF, ETFB and ETFDH) encoding ETF alpha- and beta-subunits, and ETF-QO respectively is carried out by PCR, subcloning and sequencing analysis of the patient's cDNA.

Results: The genetic analysis showed some splicing instability of the ETFDH gene. Beside the normal size of ETFDH transcript, the patient's cDNA also contains 2 shorter transcripts, 1 with skipping of exons 2 and 3 and another 1 with skipping of exon 5. There is no other gross rearrangement observed on the other 2 genes, ETFA and ETFB.

Conclusion: Further investigation is ongoing to confirm whether this splicing alteration is the sole cause or whether there is any other mutation/s contributing to the cause of the disease.

II071/MP

Are There Differences in Drug Allergy Between the Sexes?

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Aim: To determine if there are differences in the clinical features of drug allergy in males and females.

Methods: We studied the incidence of new drug allergy reactions in patients who were admitted to Tan Tock Seng Hospital over a 57-month period from 1 December 1997 to 31 August 2002. We identified these patients with the prospective Inpatient Drug Allergy Reporting system, which is administered by us and has been demonstrated to be accurate and reliable. The patients' biodata, comorbidities, types and severity of allergic drug reactions and outcome were compared between the 2 sexes.

Results: Over the study period, 161,614 (58%) male and 117,030 (42%) female patients were admitted. Among these were 286 male (47%) and 325 female (53%) patients who developed drug allergy ($p = 0.0$ by Fisher's exact test). The mean age of the male patients was 54.61 ± 18.13 years and that for females was 57.41 ± 19.86 years ($p = 0.071$). The clinical features of drug allergy were similar. The types of drugs implicated were generally the same, but reactions to anti-convulsant tend to be more prevalent in female patients. The prevalence of polypharmacy was not different between the sexes. There were 7 deaths in males patients and 5 deaths in female patients attributed to drug allergy ($p = 0.232$).

Conclusion: Hospitalised female patients are statistically significantly more likely to develop drug allergy than males. The explanation is not apparent from this study. However, there were no differences in the clinical features of drug allergy between the sexes.

II072/MP

A Study of 73 Cases of Anaphylaxis Referred to a Clinical Immunology/Allergy Service in Singapore

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Aim: To study the clinical features and aetiologies of consecutive outpatients with anaphylaxis.

Methods: A retrospective review of 73 consecutive patients who presented between 1 July 1998 and 28 February 2002. Anaphylaxis was defined as a severe life-threatening systemic IgE-mediated hypersensitivity reaction. Hypotension and bronchospasm were not required to make a diagnosis. The aetiology was determined from clinical history, Pharmacia® CAP allergen-specific IgE levels ≥ 0.7 kUA/L, and/or skin prick test (SPT) wheal diameter ≥ 3 mm using GREERS® allergen extracts or prick-prick test with the fresh/canned food product.

Results: Anaphylaxis comprised 8.2% of referrals to our outpatient service. The mean age was 30.9 ± 12.3 years (range 6-57). The main causes of anaphylaxis were food (36, 49.3%) and insect stings (22, 30.1%). Fifteen (20.5%) were idiopathic. None were due to drugs or latex. Crustaceans and mollusks caused 30% of food allergies. Honey bee and wasp caused 50% of insect sting allergies. An average of 1.97 ± 1.61 episodes of anaphylaxis occurred before the patients sought treatment. The most common manifestations were dyspnoea (58.9%), urticaria (57.5%) and angioedema (46.6%). Hypotension was documented in only 19 (26.0%) cases. A history of atopy was present in 32 (43.8%) patients, of whom 50% had asthma.

Conclusion: Food (crustaceans and mollusks) was the most common cause followed by insect stings. The inability to identify the causative insect in 50% with sting allergy limited the role of specific immunotherapy. Compared to other reported series there were no cases of drug or latex anaphylaxis.

II073/MP

The Spectrum of Allergic/Immunologic Diseases from a Clinical Immunology/Allergy Clinic in Singapore

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*Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital, Singapore***Aim:** To describe the spectrum of allergic/immunologic diseases seen in a Clinical Immunology/Allergy (CIA) clinic.**Methods:** This was a retrospective review of 889 consecutive cases referred to a CIA clinic in Tan Tock Seng Hospital for possible allergic or immunological disorders during the study period 1 January 1998 to 28 February 2002.**Results:** The mean age of patients referred was 38.6 ± 18.0 years (range 5-88); 54.8% were males and 45.2% females. The most common referrals were for adverse reactions to drugs (32.4%), airway allergy (28.1%) and urticaria/angioedema (26.1%). Drug allergy comprised 16.5% and idiosyncratic reactions to non-steroidal anti-inflammatory drugs 13.9% of all referrals. Allergic rhinitis and asthma (airway allergy) comprised 16.9% and 8.3% of all referrals, respectively. Atopic eczema/dermatitis syndrome (8.5%), anaphylaxis (8.2%), food allergy (5.3%), insect venom allergy (3.5%) and primary immunodeficiency (PID) (0.8%) were less common. The main causes of anaphylaxis were food (49.3%) and insect stings (30.1%) with none from drugs or latex. Crustaceans, mollusks and bird's nest accounted for the majority of cases of food allergy. Honey bee and wasp stings accounted for 50% of insect venom allergy. Antibody deficiency was the most common PID with chronic sinusitis and recurrent pneumonia being the most common presentation.**Conclusion:** Adverse reactions to drugs, urticaria/angioedema and airway allergy were the most common referrals to our service.

II074/MP

Long Term Outcome of a Structured Desensitization Programme for the Management of Cotrimoxazole Allergy in HIV-positive PatientsBernard THONG, Khai Pang LEONG, Chwee Ying TANG, Hiok Hee CHNG
*Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital, Singapore***Aim:** To evaluate the long term outcome following successful desensitisation to cotrimoxazole in HIV-positive patients for the prophylaxis and treatment of *Pneumocystis carinii* pneumonia (PCP) and toxoplasmosis.**Methods:** The long term outcome of 24 HIV patients successfully desensitized to cotrimoxazole was retrospectively evaluated.**Results:** Cotrimoxazole was stopped in 17 (88.3%) patients following successful desensitization for the following reasons: recurrence of drug eruption (23.5%), absolute CD4 count $> 200/\text{ml}$ following anti-retroviral therapy (ART) and hence no further need for prophylaxis (23.5%), defaulted treatment (47.1%) and cotrimoxazole failure and need for pentamidine following recurrent PCP infection (5.9%). Among 16 patients who stopped cotrimoxazole after a known period of time, the mean duration of cotrimoxazole use was 88.9 ± 74.1 weeks (8.1 – 241.6). The duration of use in the remaining patient was unknown. Of the 4 patients in whom rash occurred, none resulted in systemic hypersensitivity reactions. None of the patients with recurrence of rash or who defaulted treatment were referred back for desensitization as they were not agreeable to the procedure. Of the remaining 7 patients who continued cotrimoxazole, 5 have died of advanced AIDS or opportunistic infections not due to PCP or toxoplasmosis.**Conclusion:** The majority of patients who were successfully desensitized eventually stopped cotrimoxazole for various reasons, with less than a quarter due to recurrence of cotrimoxazole allergy.

II075/MP

A Comparative Study of Early Rheumatoid Arthritis during 1995-1997 and 2000-2002 in a Tertiary Rheumatology Centre

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*Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital, Singapore***Aim:** To compare the demographic, clinical and radiographic features of

patients with early rheumatoid arthritis (ERA) during 2 time periods (1995-1997 [group 1] and 2000-2002 [group 2]).

Methods: The demographic profile, functional status, initial presentation and radiographic features of patients with ERA were compared using a standard protocol. All patients fulfilled the 1987 American College of Rheumatology (ACR) criteria and had disease duration < 2 years.**Results:** The study population comprised of 169 patients in group 1 and 179 patients in group 2. The mean age at presentation (48.7 vs 49.6 years) and ethnic distribution were similar in the 2 groups. Female and Chinese patients predominated the study populations. The duration of illness before diagnosis was significantly shorter in group 2 (median of 8 months vs 4.4 months, $p < 0.001$). The prevalence of 5 of the ACR criteria were similar between the 2 groups. However, significantly more patients in group 1 had erosions or osteopaenia on hand radiographs (64.2% vs 29.1%, $p < 0.001$). Patients in group 1 also had significantly poorer ACR functional class at presentation (class 1: 8% vs 68% $p < 0.001$, class 2: 79% vs 23% $p < 0.001$).**Conclusion:** Comparing the 2 periods, patients with ERA were presenting earlier to our centre in recent years with not only a shorter duration of symptoms before diagnosis but also significantly less radiological damage and better functional class. These findings confirmed the importance of earlier diagnosis and treatment of RA.

II076/MP

Evaluating the Published Evidence for the Efficacy of Continuing Medical Education in Radiation Oncology

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II077/MP

Can Custom-made Biomechanic Shoe Orthoses Prevent Problems in the Back and Lower Extremities? A Randomised, Controlled Intervention Trial of 146 Military ConscriptsKristen LARSEN¹, Charles BAYCROFT¹, Flemming WEIDICH², Charlotte LEBOEUF-YDE¹¹The Medical Research Unit, Ringkjøbing County, Ringkjøbing, Denmark, Ringkjøbing, Denmark, ²Jutland Dragoon Regiment, Holstebro, Denmark**Aim:** To investigate if biomechanic shoe orthoses can prevent problems of the back and lower extremities and if reducing the number of days off-duty because of back or lower extremity problems is possible.**Methods:** Health data were collected by questionnaires at initiation of study and 3 months later. Custom-made biomechanic shoe orthoses to be worn in military boots were provided to all in the study group during the 3-month

intervention period. No intervention was provided for the study group. Differences between the 2 groups were tested with the chi-square test, and statistical significance was accepted at $P < 0.05$. Risk ratio (RR), risk difference (ARR), numbers needed to prevent (NNP) and cost per successfully prevented case were calculated.

Results: Results were significantly better in actual-use analysis in the intervention group for total number of subjects with back or lower extremity problems (RR 0.7, ARR 19%, NNP 5, cost US\$98); number of subjects with shin splints (RR 0.2, ARR 19%, NNP 5, cost US\$101); number of off-duty days because of back or lower extremity problems (RR 0.6, ARR <1%, NNP 200, cost US\$3750). In an intention-to-treat analysis, a significant difference was found for only number of subjects with shin splints (RR 0.3, ARR 18%, NNP 6, cost US\$105), whereas worst-case analysis revealed no significant differences between the study groups.

Conclusion: It may be possible to prevent certain musculoskeletal problems in the back or lower extremities among military conscripts by using custom-made biomechanic shoe orthoses.

II078/MP

Implementation and Audit of a Pain Management Protocol in a Nursing Home

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Aim: To improve pain management in residential care services

Methods: A cross sectional survey of medical records pre and post-implementation of a new pain protocol in a 75 bed nursing home in Victoria. The project involved the development of a new pain policy and protocol through an abbreviated literature review. The existing pain policy, procedures and assessment tools of the nursing home were replaced with a new pain management decision support algorithm, all staff were trained and a senior nurse pain champion was enlisted. A post-implementation audit of changes in pain assessment, diagnosis and management was undertaken 4 months later.

Results: The prevalence of patients in active pain (defined as documented evidence of pain or the use of analgesia within the last 2 weeks) was similar in pre and post-implementation of protocol. Implementation of the protocol resulted in a significant: i) increase in diagnoses for the cause of active pain ($p < 0.000$), ii) reduced use of multiple analgesics ($p < 0.000$), iii) reduced prescription of prn paracetamol ($p < 0.005$), and iv) reduced use of potentially harmful analgesics.

Conclusion: Persistent pain is a very common and challenging issue for residential care providers. Individual care providers can enhance the quality of pain management by implementing an individually targeted pain guideline.

II079/MP

Secretion of Defensins From Human Conjunctival Cells Upon LPS Stimulation

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Aim: To investigate the secretion of defensin by human conjunctival cells

Methods: Human conjunctival cells were isolated and cultured in vitro. Cells were stimulated by 20 ng/ml lipopolysaccharide (LPS). Cell culture mediums were collected at different time points after treatment and subjected to liquid chromatography-mass spectrometry (LC-MS) analysis for the detection of defensins. Expression of defensin genes were also analysed by RT-PCR. Defensin stimulation of conjunctival cell proliferation was analysed by BrdU incorporation assay.

Results: Message for human defensin beta 1 and 2 mRNA was found in quiescent cultured conjunctival cells by RT-PCR but no defensin protein was found in the culture medium. 7 hrs after LPS stimulation, defensin beta 1 was detected in the culture medium by LC-MS analysis. However, BrdU incorporation was minimal suggesting little effect of secreted defensin on conjunctival cell proliferation.

Conclusion: The secretion of defensins by human conjunctival cells upon

LPS stimulation provides the evidence of innate immune response.

II080/NUR

Reducing Hospitalisation for Acute Exacerbation of Chronic Obstructive Pulmonary Disease with a Homecare Programme

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Aim: To evaluate the early outcomes of a nurse-led, post-discharge home care programme for patients following hospitalisation for acute exacerbation of chronic obstructive pulmonary disease (COPD).

Methods: Patients were recruited before discharge from hospital and received home visits by trained nurses, telephonic support with rapid access to an Acute Respiratory Assessment Service (ARAS) during the early post-discharge period. Outcome measures were hospitalisation days and hospital readmissions.

Results: Preliminary findings of 42 patients (mean age 70 years, 2 female, mean FEV1 36.7 percent predicted) showed that the mean length of hospital stay was reduced from 9.7 days to 6.7 days in the 6-month period before and after the programme ($p = 0.152$). The number of readmissions (within 1 month of hospital discharge) was reduced from a mean of 2.7 to 1.4 episodes ($p = 0.001$).

Conclusion: This comprehensive, post-discharge home care programme reduced the number of hospital readmissions and hospitalisation days in patients with acute exacerbation of COPD.

II081/NUR

Prevalence of Postprandial Hypotension in the Elderly

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Aim: Background postprandial hypotension is a common disorder of blood regulation in the elderly who are aged 65 years and above. Syncope and falls may often result from this condition. We sought to explore if there was an association between meal ingestion and a drop in systolic blood pressure among the elderly in-patients of Woodbridge Hospital.

Methods: The objective of the study was to determine whether there is a drop in the blood pressure after a meal in the elderly. 80 male and 104 female elderly inpatients ($n = 184$), aged 65 years and above, were studied. Blood pressure was monitored half hour before and 3 hourly after breakfast and half hour before and 3 hourly after lunch.

Results: Of the 184 subjects, 98 of them had reduction in their systolic blood pressure post breakfast and 72 had a drop post lunch for the 1st hour post meal. The systolic blood pressure in subjects taking full diet was likely to drop >20 mmHg than those with other meals at 1 hour after breakfast ($p = 0.010$, OR = 1.08, 95% CI 1.02 to 1.15, Fisher's exact test). There was a significant reduction in systolic blood pressure >10 mmHg among subjects aged 90-100 years at 1 hour after breakfast ($p = 0.019$). There was a statistically significant difference between those with and without anti-hypertensive medication ($p < 0.001$). The findings were consistent over the 3 hours post breakfast as well as post lunch.

Conclusion: All health care personnel caring for the elderly should be aware of the hypotensive effects of food intake in the elderly and should consider postprandial hypotension in the evaluation of syncope, falls and dizziness.

II082/PD

A Pilot Study on Developing a Group Cognitive Behavioural Therapy for Children with Anxiety in Singapore

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Aim: Recent treatment studies conducted overseas have demonstrated the efficacy of group cognitive behavioural therapy for anxious children. Given the cultural differences, we decided to refine a manualised group cognitive

behavioural treatment designed for North American children specifically for use with our children. The objective of this pilot study was to provide preliminary support for this treatment.

Methods: Eleven children aged 8 to 12 years from the Child Guidance Clinic with diagnosed anxiety disorders were recruited for the study. The child's anxiety scores were assessed using a self-reported measure, the Multidimensional Anxiety Scale for Children (MASC) and a parent-reported measure, Child Behavioural Checklist for Children (CBCL) on 3 time-periods: pre-treatment (T1); post-treatment (T2) and 3 months post-treatment (T3). All 11 children completed the 8 1-hour sessions of treatment.

Results: The MASC total anxiety scores were significantly reduced after treatment and these treatment gains were maintained after 3 months at $p < 0.05$. However, there was no significant reduction in the Total Internalizing Problems scores on the CBCL even though one of the subscales scores, Somatic Complaints were significantly lower after treatment at $p < 0.05$.

Conclusion: Even though the parents did not perceive their children to be less anxious after treatment, the children rated themselves as being less anxious after treatment and on 3-months follow-up. This study provides some preliminary support for the efficacy of our manualised group cognitive behavioural treatment.

II083/PD

A survey of Drug Information Service (DIS) in Singapore

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Aim: This survey aims to evaluate the workload, staffing and expenditure of Drug Information Centres (DICs).

Methods: A questionnaire was sent to all DICs in Singapore in October 2002. Of the targeted DICs, 83% responded.

Results: All the respondent DICs in Singapore only operate during office hours. These DICs are staffed by 1 full time Drug Information (DI) pharmacist and 1 trained covering staff. Eighty percent of the respondents felt that more than 1 DI pharmacist is required for better provision of services. The average number of enquiries received per month was 187 (61-349) from January 2001 to September 2002. The average number of drug reviews prepared per month was 2.9 (1.1-4.4) from January 2001 to September 2002. DI pharmacists prepared most of the reviews except for TTSH where pre-registration pharmacists prepared most of them with subsequent vetting by the DI pharmacist. The average number of pre-registration pharmacists and master students (MPharm) trained was 6.5 (0-14) for year 2001 and 2002. The average expenditure on DI references (including journals and databases subscriptions) was S\$27,212 (S\$8,607-S\$55,300) for year 2002.

Conclusion: The staffing level appears to only cater for a basic routine service with workload and expenditure varying with the level of activity. There are altogether 6 DICs in Singapore but no provision of a comprehensive islandwide facility. Having either a coordinated or alternatively a centralised DIS could be a way to resolve besides allowing for longer operating hours, savings on DI references and enhancing the level of such DI services.

II084/PD

Respiratory Histology and Cytology in Tan Tock Seng Hospital

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Aim: Cytology is a rapid, cost-effective and reasonably accurate investigation modality for diagnosis of lung lesions. We seek to characterise the diagnostic profile of various cytological techniques and assess their performance with histology as the gold standard.

Methods: The results from the various cytology specimens received in our department from 2000 to 2002 were gathered and correlated with available follow-up histology.

Results: From the 2318 cytology samples received, 94 cases of transbronchial needle aspirations (TBNA) and 288 cases of percutaneous fine needle aspiration cytology (FNAC) had subsequent histology available. 25 TBNA

samples (25.8%) and 31 FNAC samples (8.2%) were found to be unsatisfactory for evaluation. Most of these cases had scant cellularity and yielded mainly blood. The diagnostic sensitivity for malignancy is 72.2% for TBNA and 91.7% for FNAC. The diagnostic specificity is 90.9% for TBNA and 91.8% for FNAC. Similar data will be calculated for the other cytological techniques and a review undertaken to determine the causes of histo-cytological non-correlation. A variety of respiratory infections were also diagnosed on cytology, among which are tuberculosis, cryptococcal, cytomegalovirus and paragonimus infections.

Conclusion: Our results show a reasonably high standard of respiratory cytology performance in our department. It also reaffirms that cytology samples from the lung are useful alternatives to more invasive surgical methods in the diagnosis of lung cancer. Cytology is helpful in the work-up of lung infections, including opportunistic infections in immunocompromised patients. Lastly, the importance of good clinical and radiological-pathological correlation is crucial for optimum patient management.

II085/PD

Fine Needle Aspiration Cytology of Breast Lesion in Tan Tock Seng Hospital, Singapore

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Aim: Carcinoma of the breast is one of the most frequent cancers reported amongst females in Singapore. Tan Tock Seng Hospital Singapore, has reported between 92 and 127 new cases of breast cancer each year over the 5-year period of 1996 to 2000. In this study, we measure the predictive value of the test, and the spectrum of diseases that this procedure is used for.

Methods: All cases of fine needle aspiration of the breast and their follow-up biopsies received from January 1996 to December 2000 were retrieved and reviewed.

Results: A total of 465 fine needle aspirations were received within the studied period. 316 cases has at least 1 follow-up biopsy; 76 of which confirmed the malignancy diagnosed by the fine needle aspiration with 100% positive predictive value. Most of carcinoma cases were diagnosed in women aged between 40 to 49 years that formed the largest group of patients.

Conclusion: With a high positive predictive value, fine needle aspiration is being increasingly accepted as pre-operative diagnostic tool for breast lesions in this institution.

II086/PD

Idiopathic Granulomatous Mastitis: Diagnosis and Management

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Aim: To review the findings in 9 patients diagnosed with idiopathic granulomatous mastitis (IGM) at National University Hospital, with particular attention to imaging findings.

Methods: A retrospective review was conducted of 9 women (age range 29-53 years, mean age 35.8 years, median age 41 years) presenting to our institution between 1997 and 2001, who were diagnosed with IGM after histological analysis. Imaging findings, clinical features at presentation and subsequent outcome were reviewed.

Results: All patients had either discretely palpable lumps or diffuse palpable breast thickening. The lumps ranged in size from 2 to 10 cm. All patients had sonographic examinations; 7 patients had mammographic studies and 2 patients had supplementary magnetic resonance imaging (MRI). MRI findings in IGM have been previously described only once before. Mammographic features varied from asymmetric density to architectural distortion, nipple retraction, stromal thickening and partly circumscribed nodules. One patient had diffusely dense breasts with no focal lesion identified. At sonography, 8 patients had hypoechoic focal masses with variable borders in different cases. MRI was performed in 2 patients using a GE Signa 1.5T MRI unit. The appearances and enhancement pattern were atypical for carcinoma. None had

acid-fast bacilli on histology, and none were diagnosed as having breast tuberculosis on culture. All patients responded well to treatment, regardless of the method used.

Conclusion: IGM is an uncommon clinical entity which can be confused with carcinoma. Imaging features with mammography and sonography are non-specific. MRI scans were atypical for carcinoma and were consistent with inflammation.

II087/PD

In-Patients' Satisfaction and Compliance of Therapeutic Diets in National University Hospital, Singapore

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Aim: A therapeutic diet involves modification of a normal diet to meet the patients' specific needs, in order to treat various diseases and disorders. Patients' satisfaction and compliance with therapeutic diets significantly affect their nutritional status and disease control. However, therapeutic diets often present food acceptance problems due to multi-factorial reasons. In this study, factors affecting in-patients' satisfaction and reasons behind non-compliance were investigated.

Methods: 300 adult in-patients on therapeutic diets in NUH were randomly selected in a 1-month period. A questionnaire was designed to collect qualitative data of 4 areas: socio-demographic; foodservice quality; diet compliance; and dietetic intervention. Data were collected through semantic differential scale of 3-point and 5-point, 'close-ended' and 'open-ended' questions. A pilot study was carried out for verification.

Results: Overall meal satisfaction was shown to be positively and significantly affected by overall food quality ($r = 0.602$, $P < 0.05$), especially by taste, freshness, temperature and variety. Other factors affecting meal satisfaction include accuracy of meal served ($f(2,297) = 4.67$, $P < 0.05$), belief in diet control ($f(1,298) = 6.07$, $P < 0.05$) and attitude of staff taking meal orders ($r = 0.254$, $P < 0.05$) and serving meals ($r = 0.339$, $P < 0.05$). After dietetic intervention, in-patients were more likely to follow dietary instructions (Cramer's $V = 0.260$, $P < 0.05$) and comply with therapeutic diets (Cramer's $V = 0.414$, $P < 0.05$).

Conclusion: In-patients' satisfaction and compliance of therapeutic diets can be enhanced by improving food quality and staff attitude; and through providing adequate and accurate dietetic information and intervention.

II088/PD

Triple Immunolabelling Using Combined Immunoperoxidase and Immunofluorescence Techniques on Paraffin Embedded Tissue Sections

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Aim: We describe a method to demonstrate expression of multiple antigens using a triple immunolabelling technique in paraffin embedded tissue sections.

Methods: 4-um thick tissue sections are cut from paraffin embedded tissues. Following de-waxing and rehydration, they are subjected to heat-induced antigen retrieval. Immunoperoxidase staining using the first antibody is performed, followed by double immunofluorescence labelling using FITC and Texas Red. Images are captured in monochrome and pasted in the blue-, red- and green-channels of an imaging programme such as Photoshop.

Results: The co-expression of up to 3 different antigens may be demonstrated in various types of tissue sections.

Conclusion: Triple immunolabelling can be reliably performed on paraffin embedded tissue sections using a combined immunoperoxidase and immunofluorescence technique.

II089/PD

Prealbumin: Nutritional Predictor of Outcome and Length of Hospitalisation in Patients Under Intensive Care

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Aim: Prealbumin (PA) is a visceral protein and is decreased in patients suffering from malnutrition and systemic stress. Since malnutrition and degree of systemic stress determine rate of mortality and recovery of patients, PA can also correlate with patients' outcome and length of hospital stay.

Methods: Blood samples from 20 Intensive Care Unit patients with various clinical diagnoses, sent to our laboratory, were randomly selected and serum PA measured on Beckman Coulter IMMAGE Immunochemistry System. PA levels on admission and during hospitalisation were then correlated to their outcome and length of hospitalisation.

Results: Group1: 6 patients with initially normal PA of >18 mg/dL maintained levels above 10 mg/dL were hospitalised between 8-15 days and well on discharge. Group2: 5 patients with initial PA of 11-18 mg/dL; 2 with improved PA level survived with hospital stay of 9-31 days, 3 with 20% decline from their initial PA level, hospitalised for 26-60 days, did not survive. Group3: 8 patients with low PA of <10 mg/dL on admission; 5 with improved PA level survived with hospital stay of 26-74 days and 3 with declined PA level, hospitalised for 11-49 days, succumbed to illness. One patient with initial PA of 9.6 mg/dL with little increase in PA level, did not survive after 8 days of hospitalisation.

Conclusion: In this short study, we found that patients with initial PA levels <10 mg/dL were hospitalised longer compared to patients with PA levels >10 mg/dL. The decline of PA levels during hospital stay can also predict outcome in ICU patients. Hence, PA with its small body pool and short half-life is a useful and sensitive marker of recovery and deterioration of patients and initiation of treatment intervention.

II090/PD

BioRad D-10: A New Small Laboratory HPLC HbA1c Analyser

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Aim: To study the measurement of glycated haemoglobin (HbA1c) on BioRad D-10 (BioRad Laboratories, USA), a new small laboratory high performance liquid chromatography (HPLC) instrument, and compare its performance to our current analyser DCA 2000 (Bayer Laboratories, USA), which measure HbA1c by inhibition agglutination.

Methods: 1) To measure HbA1c on EDTA coagulated whole blood samples from our outpatient clinics. 2) To study the imprecision of D-10 using 2 levels of BioRad quality control material and 2 levels of pooled EDTA whole blood samples.

Results: Linear regression data of HbA1c were: $DCA\ 2000 = 0.99 (D-10) + 0.29$, $R = 0.97$, $n = 75$. The bias plot showed -0.2% (range -0.8% to 0.5%) lower %HbA1c results on D-10 when compared to DCA 2000. On D-10, within day imprecision coefficient of variation (CV) was $<1.0\%$ for HbA1c range from 5.4 to 10.5% ($n = 20$) and between days imprecision ($n = 9$) was $CV \leq 1.6\%$. On DCA 2000, between days imprecision was $CV\ 4.6\%$ and 5.8% ($n = 15$) for HbA1c control of 5.5% and 9.9%, respectively.

Conclusion: The D-10 showed excellent precision whereas DCA 2000 did not meet the HbA1c analytical performance criteria ($CV < 3.0\%$) for diabetic monitoring. The D-10 can run up to 10 samples per rack in 36 minutes when compared to 5 samples on the DCA 2000, thus offering a more efficient same session outpatient setting. The HPLC results are reported in a trace format where abnormal haemoglobin patterns may be viewed to determine accuracy of the results. However, operator training is required to maximally utilise this HPLC instrument that can be traceable to the National Glycohemoglobin Standardisation Program (NGSP).

II091/PD

Evaluation of an Inpatient Group Counselling Programme for Patients with Hyperlipidaemia

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Aim: To evaluate patients' satisfaction of an inpatient group-counselling programme and its effectiveness in increasing their nutritional knowledge.

Methods: An inpatient group-counselling programme on "Cholesterol and Diet" was developed and conducted by the dietitians for patients and caregivers. Patients referred to the dietitians for cholesterol lowering advice were screened for suitability and scheduled to attend a 1-hour session. PowerPoint presentation and printed materials were used to support the verbal information. Evaluation of nutritional knowledge from the participants was obtained at the beginning and at the end of the session. Post-session feedback on patients' satisfaction was also conducted.

Results: 114 patients and caregivers completed the programme. 57% agreed that the programme met most of its objectives and 33% agreed that all its objectives were met. 43% rated the session useful, 44% rated it very useful. 66% found the presentation clear and easy to follow, 23% found it excellent. 25% rated the dietitian excellent in sustaining their interest, 69% rated as good. 92% found the duration just right. 49% of the patients preferred the group session whereas 32% preferred both group and individual sessions. 93 patients and caregivers completed the nutritional knowledge test. 21 were excluded due to incomplete questionnaires. Paired t test showed a significant improvement in nutrition knowledge scores after attending the programme ($P < 0.01$).

Conclusion: The group-counselling programme was effective in improving the nutrition knowledge in cholesterol lowering among the patients and caregivers. A high patient satisfaction to the programme also supported the continuation of this new service.

II092/PD

Identifying the Need for a Pharmacist Run Epilepsy Clinic in Singapore

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Aim: To determine the knowledge level of Asian patients with epilepsy regarding their condition, the importance of information provision, quality of life (QOL) and the feasibility of setting up an epilepsy clinic managed by pharmacists in a local hospital.

Methods: Patients with epilepsy were interviewed using a questionnaire consisting of the QOLIE-10; and questions to determine their seizure summary, knowledge level and level of importance of providing information regarding their condition, lifestyle treatments and anti-epileptic drug therapy. Student's t test and ANOVA were used for statistical analysis.

Results: Fifty-one patients (mean age $\pm$ SD, 34.6 ± 11.9 years) were studied. QOLIE-10 scores between patients who had 10 or less and more than 10 seizures per year were significantly different. Patients who had more than 10 seizures per year had a poorer expressed QOL (26.2 ± 3.51). Patients on polytherapy experienced more adverse events. Common side effects included drowsiness, gastrointestinal side effects, memory difficulties and insomnia. Most patients gave a "none" response to their level of knowledge regarding epilepsy (54.9%), lifestyles and social impact (49.0%), and anti-epileptic drug treatment (64.7%). Provision of information about drug therapy was rated as the most important, followed by lifestyles and social impact, then medical knowledge. Most were willing to pay for pharmacists' consultations. Those who were unsure or unwilling to pay felt that their seizures were already adequately controlled or had time and monetary constraints.

Conclusion: There is an information deficit in Asian patients with epilepsy. An epilepsy clinic managed by pharmacists should be set up in the hospital for a trial period.

II093/PD

The Beneficial Effects of the Joint Protection Course on the Joint Protection Knowledge of Arthritis Patients After 6 Months

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Aim: General knowledge with regard to joint protection is usually poor amongst patients suffering from arthritis. The occupational therapist conducted a 4-week course which aim to improve the knowledge of these patients with regard to joint protection. We have undertaken a study to determine the effectiveness of such a course in improving hand functions as well as helping them to protect their joints from further damage.

Methods: The subjects were interviewed using joint protection knowledge assessment questionnaire a week before the course starts and 1 month after the course. The same set of questionnaire was administered 6 months after the course. Other scores for quality of life measurement (Short Form 36), Rheumatology Attitude Index (RAI) and disability scores were also measured.

Results: A total of 50 participants completed all the questionnaires. Comparing the joint protection knowledge score at pre-course and immediately after the course showed that there was no difference (19.0 vs 24.2). There was also no difference in score when we compared pre-course and 6 months post-course (19.0 vs 22.4). More participants are being enrolled and analyses of the data will be reported in October 2003.

Conclusion: Based on the preliminary analysis of the of 50 participants, the joint protection course does not appear to have significant effect on the joint protection knowledge of arthritis patients.

II094/PD

Botulinum Toxin A in the Management of Upper Extremity Spasticity in Children with Cerebral Palsy: A Systematic Review

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Aim: The purpose of this paper was to obtain a review of literature regarding the use and efficacy of BTX injections to improve upper extremity function in children with cerebral palsy. It also seeks to identify occupational therapy evaluation and management options for children with cerebral palsy receiving BTX injections to their upper extremity.

Methods: The study design consists of a systematic review. A computer-aided search was conducted using the databases of Medline and Cinahl. The reports were also located through related readings and the reference lists of the articles. Any studies investigating the use of botulinum toxin A in upper extremity spasticity were included in the analysis.

Results: Principal studies concur that BTX treatment is safe and effective in reducing spasticity. Instruments used to assess for improvement following the injections include the Ashworth scale for measuring spasticity and goniometric measurements. However, there is a paucity of literature regarding the long-term functional outcome following BTX injection. There is also insufficient detail in the studies to guide therapeutic intervention for children with cerebral palsy receiving BTX injections for upper extremity spasticity.

Conclusion: The studies reviewed do not have sufficient detail to allow repetition of the therapeutic management treatment protocol by other researchers. The review also demonstrated the need to undertake further research into the efficacy of BTX in the management of upper limb spasticity in cerebral palsy, including impact of longer term use and functional outcome following botulinum toxin A injections.

II095/PD

Identification of Differentially Expressed Genes Induced in BCG Treatment of Bladder Cancer

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Aim: Mycobacterium bovis BCG (Bacillus-Calmette Guerin) has been effectively used as an immunomodulatory agent for bladder tumour. However,

its molecular mechanisms of therapy has not yet been elucidated and we are only beginning to understand its immune mechanisms. We aim to search for differentially expressed genes correlating to bladder tumour killing.

Methods: Representational differential analysis (RDA), as described by Pastorian et al, was performed to isolate differentially expressed cDNA sequences from cultures of control and BCG treated MGH cells. The fragments were then cloned using the PGEM-T vector kit and sequenced. RNA samples from other cell lines were hybridised using some of the fragments as probes.

Results: Differentially expressed fragments were isolated in the third subtractive hybridisation round. Using some of the fragments as probes, differential expression was confirmed by hybridizing with RNA samples from other bladder cell lines. Sequence similarity searches with GenBank were performed using the BLAST algorithm. Some of the sequences were from known genes such as housekeeping genes and a few unique genes were obtained.

Conclusion: This method enables the identification of genes that are differentially expressed.

II096/SOGD

Pulmonary Infarction Secondary to Tumour Embolism From Lower Limb Osteosarcoma

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Aim: To describe radiological findings in a patient with osteogenic sarcoma presenting with pulmonary embolism.

Methods: A 14-year-old Chinese boy presented with a 1-week history of breathlessness and right knee swelling of 4 months duration. He was investigated with urgent CT pulmonary angiography. The right knee swelling was investigated with plain X-ray, Doppler, bone scan and MRI. He was followed up with CT scan.

Results: CT pulmonary angiography demonstrated emboli in the right main pulmonary artery and segmental and sub-segmental branches. Opacities in the right lung compatible with pulmonary infarcts were also noted. Doppler ultrasound performed on the same day showed thrombosis of the right superficial femoral vein. A filter was placed in the inferior vena cava to prevent further embolisation. CT scan done after the IVC filter demonstrated increase in the size of emboli with further occlusion of the pulmonary arterial tree. He was treated with urgent surgical embolectomy. Plain X-ray, bone scan and MRI of the right knee were consistent with an osteosarcoma. A biopsy of the tumour confirmed the diagnosis. The patient was treated with chemotherapy and above knee amputation of the right lower limb. Follow-up CT scans demonstrated progression of the disease with development of pulmonary metastases. The patient succumbed to the disease 8 months after initial presentation.

Conclusion: Pulmonary tumour embolism is difficult to diagnose in patients without known malignancy. CT scan is useful in detecting tumour embolus, and in follow-up and management of the case.

II097/SOGD

Delayed Treatment of 5th Carpometacarpal Joint Dislocation in Association With 4th Metacarpal Fractures

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Aim: Dislocations of the ulnar carpometacarpal joints (CMCJ) are rare injuries. The fourth and fifth joints are more commonly affected. The diagnosis is often not made initially due to subtle clinical and radiological findings. Treatment, particularly of late cases, is controversial. We review our experience in 2 such cases of fourth metacarpal shaft fractures with fifth carpometacarpal joint dislocation.

Methods: A review of 2 cases of fifth CMCJ dislocation occurring in association with fourth metacarpal fracture is presented. The clinical presentation, diagnosis, management, clinical and radiological outcomes are discussed.

Results: Both cases occurred in young males with indirect injury to the hand. In both cases, the fracture was diagnosed but the dislocation was initially overlooked. As a result, the treatment of the dislocation was delayed. Open reduction and stabilisation of the dislocation together with corrective osteotomy to the malunited fourth metacarpal shaft was performed in 1 case, and fusion of the hamatometacarpal joint done for the second. Both patients had satisfactory outcomes.

Conclusion: A high index of suspicion is required to diagnose an ulnar carpometacarpal dislocation. An obvious fracture may distract attention from a second more subtle injury. An indirect injury resulting in a solitary fracture of the non-border metacarpal should raise the suspicion of an associated injury at the carpometacarpal joint. The findings on initial clinical examination and radiographs are often subtle. A CT scan may be useful for diagnosis and surgical planning. With reduction and adequate stabilisation, a good outcome is expected. Fusion should be performed in delayed cases with significant articular surface damage.

II098/SOGD

Flap Reconstruction Following High Pressure Injection Injuries

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Aim: High pressure injection injuries are well known to cause significant injury to the hand, with high amputation rates and poor functional outcome. Traditionally, surgical treatment has consisted of early aggressive debridement followed by secondary closure. In more severe cases, the skin of the digit may also be damaged and may need to be debrided, leaving a large skin defect which cannot be closed directly. Flaps are one surgical option for closure of this defect. We reviewed the results of cases treated with flap reconstruction by this department over the past year.

Methods: A retrospective review of 3 cases performed over the past 1 year.

Results: Salvage of the affected digits with proper skin coverage was achieved. Functional outcome was still impaired, and worse for the patient who was non-compliant with hand rehabilitative therapy.

Conclusion: Flap coverage offers a good option for reconstruction after high pressure injection injuries of the hand and can increase the chance of digit salvage, as well as give an acceptable functional and cosmetic result.

II099/SOGD

Technique of Intra-articular Loop Wiring for Bony Fixation in Finger Replantation

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Aim: Intraosseous loop wiring is 1 technique of bone fixation in finger replantation. It is relatively simple and fast to use and provides sufficient stability for early mobilisation. It is best used for injuries at the level of the mid-shaft. For injuries near the proximal interphalangeal joint, the traditional loop wiring is not suitable because there is insufficient bone to pass a second loop. An alternative method of fixation, usually involving transfixation of the joint is often required. We have modified the wiring technique to be used in such a situation. One loop of wire passes intraarticularly within the proximal interphalangeal joint (PIPJ), while the other loop passed perpendicular to it through the base of the bone.

Methods: A clinical and radiological review of 2 clinical cases of finger replantation where the modified technique is used to achieve bony fixation is presented.

Results: One patient had good preservation of the joint motion with the intraarticular wire passed between the condyles in the anteroposterior direction. The other had a limited range of motion at the PIPJ when the intraarticular wire was passed in the coronal plane across both condyles. Both fingers remained viable and no complications were seen. The implants were removed at 2 months.

Conclusion: This technique is a useful alternative for bony fixation in amputations around the PIPJ. In such situations, the traditional method of loop wiring cannot be applied. This modification is simple to perform and does not transfix the PIPJ. The replanted finger can be mobilised immediately after surgery which improves the outcome. Early removal of the wires is necessary.

II100/SOGD

Is Out of Hours Body CT Useful?

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Aim: To assess the clinical impact of out of hours body CT.

Methods: Retrospective study of 50 consecutive patients who had out of hours body CT. Indications were grouped into 5 categories: trauma, sepsis, suspected pulmonary embolus, acute abdomen and others. These were then divided into positive (abnormal) scans and negative studies. Incidental relevant findings were analysed.

Results: 50% (25/50) of the scans were for trauma, 16% (8/50) for sepsis, 12% (6) for query pulmonary embolism, 12% (6) for acute abdomen and 10% (5) for other indications. Positive scans which impacted management were found in 60% (15/25) of trauma, 37.5% (3/8) of sepsis, 17% (1/6) of suspected pulmonary embolism, 17% (1/6) of acute abdomen and 20% (1/4) of other cases. Relevant incidental findings were noted in 20% (5/25) of trauma cases.

Conclusion: Out of hours body CT impacts management positively but needs to be used selectively.

II101/SOGD

Phenol Burns and Toxicity

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Aim: To discuss the severe adverse effects of phenol, a corrosive aromatic alcohol used in industries, as well as present current concepts in the management of phenol burns and toxicity

Methods: A case report is discussed of a 44-year-old male who was splashed extensively by phenolic acid under pressure when the valve of the drum in which it was stored was accidentally released

Results: The patient sustained 33% severe second-degree partial thickness burns involving his face, trunk and limbs. This was complicated by ocular and respiratory burns; "dark urine" and subsequent acute renal failure; cardiac dysrhythmias and pneumonia. The patient recovered and was discharged after 18 days of hospitalisation

Conclusion: Although phenol is extremely corrosive and toxic with the potential to cause many complications as seen in this patient, with intensive supportive therapy patients can recover.

II102/SOGD

Diverticular Disease of the Colon in Singapore – An Epidemiological Study

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Aim: Diverticular disease of the colon demonstrates significant geographic and ethnic variation with regards to its prevalence and epidemiological profile. Although the disease is clinically significant in our local setting, updated local studies are lacking.

Methods: A retrospective review of 1698 consecutive barium enemas performed from January 2001 to August 2002 was conducted. Findings were assessed on their prevalence due to age, sex and racial characteristics.

Results: The overall prevalence of diverticular disease was 45.3%. Right-sided diverticular disease was more common than left-sided disease (60% vs 30%, $p < 0.05$). The prevalence of this condition increased with age, suggesting an acquired condition; but genetic factors may influence the site of development of the diverticuli. Gender distribution was equal in this study.

Conclusion: Diverticular disease of the colon in the Singapore population has a predisposition to right-sided distribution in contrast to the Western populations. Similar findings in other epidemiological studies on other Asian type populations suggest that genetic factors may influence the site of development of colonic diverticuli. In a clinical setting, an understanding of the prevalence and distribution of the disease would impact on the management of older patients presenting with right-sided abdominal symptoms.

II103/SOGD

Visual Outcome After Cataract Surgery in Diabetic Versus Non-Diabetic Patients and Also Visual Outcome of Phacoemulsification Versus Extracapsular Cataract Surgery in Diabetic Patients

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Aim: To compare visual outcome after cataract surgery of non-diabetic versus diabetic eyes and phacoemulsification versus extracapsular cataract extraction in diabetic patients.

Methods: 198 eyes of 189 patients were recruited for a retrospective study over an 11-month period with a minimum of 6 weeks follow-up. Best Snellen acuity was used to measure post-operative visual acuity. Patients with other ocular pathologies except diabetic retinopathy were excluded. All required information were obtained from the case-notes. They were grouped based on diabetic status and surgical techniques (phacoemulsification or extra-capsular cataract extraction).

Results: 49.4% of diabetic eyes achieved a visual acuity of 6/9 or better compared to 75.2% of non-diabetic eyes ($p < 0.001$). 76.5% of diabetic eyes compared to 92.3% of non-diabetic eyes achieved a visual acuity of 6/12 or better ($p < 0.005$). 30 (60%) diabetic eyes with no retinopathy achieved a visual acuity of 6/9 or better compared with only 10 (32.3%) diabetic eyes with coexisting retinopathy ($p < 0.022$). 23 (54.8%) of diabetic eyes who underwent phacoemulsification had best post-operative visual acuity of 6/9 or better compared to 19 (48.7%) of eyes who underwent ECCE (extracapsular cataract extraction) ($p = 1.000$). 34 (81.0%) of diabetic eyes achieved best post-operative visual acuity of 6/12 or better after phacoemulsification compared to 33 (84.6%) after ECCE ($p = 0.793$).

Conclusion: Patients with diabetes mellitus tend to have worse post-operative visual acuity compared to normal patients. Diabetic patients with diabetic retinopathy have poorer post-operative visual acuity compared to diabetic patients without retinopathy. Phacoemulsification or ECCE did not statistically affect the post-operative visual outcome in diabetic patients.

II104/SOGD

Laparoscopic Radical Prostatectomy: Initial Experience and Perspective from an Asian Centre

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Aim: To report the first 3 laparoscopic radical prostatectomy (LRP) done in Singapore, highlighting the hurdles and difficulties surmounted and comparing the results to reported Western and Japanese figures.

Methods: The first 3 LRP (Montsouris technique) done were reviewed critically with respect to the following: selection of cases, setting up of laparoscopic equipment, readiness of surgeons and assistants, operative approach, operative time, blood loss, hospital stay, post-operative recovery, functional recovery, final pathology and cancer control. Difficulties are noted and suggestions on improving operative time and outcome of future LRP are made.

Results: Average operative time was 9 hours. Average length of stay was 3 days. There was no intra-operative complications. Average blood loss was 400 mL. One patient had an anastomotic leak requiring catheterisation for 4 weeks. All 3 patients are continent. Results are comparable to initial American and recent Japanese series. Difficulties encountered included difficulty in identifying tissue planes, limited dexterity, intra-corporeal suturing and untrained scrub nurses and assistants. Suggestions to improving subsequent cases include training of assistants and scrub nurses with videos, training on simulators and using robot assistance.

Conclusion: Initiating a LRP programme in Asia is an uphill task. From an Asian perspective, the difficulties of performing an LRP are chiefly related to the lack of localised prostate cancer amenable to surgery. However, with the anticipated increasing number of prostate cancer and a stage migration towards early prostate cancer, more and more LRP will be done in the near future in Asia.

II105/SOGD

Strategies to Minimise Cost in Laparoscopic Appendectomy

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Aim: Laparoscopic appendectomy allows better exploration and lavage of the abdominal cavity, resulting in lower intra-abdominal abscess rates, better cosmesis and in some, reduction in hospital stay. However, it has not been widely practised as the use of certain instruments adds significantly to cost. In addition, laparoscopic expertise is required. In this review, the feasibility of using certain cost conserving techniques by a surgeon in advanced surgical training was assessed.

Methods: A personal review of 35 cases of laparoscopic appendectomy was conducted. The technique involves strategic placement of the 2 5-mm instrument ports; 1 at the suprapubic region well hidden by the pubic hair, the other at the right iliac fossa at the level of the umbilicus. Intracorporeal knot tying was used instead of the usual instrumentation such as stapling devices.

Results: Three minor complications were observed. One patient had subcutaneous emphysema around the right iliac fossa. The other 2 patients had umbilical port infection, which required only outpatient management. The cases with wound infection occurred in the group of patients where the appendix was delivered through the camera port without the use of an endobag. No infection was observed when a bag was used.

Conclusion: Laparoscopic appendectomy is a feasible technique for surgeons who have completed their basic surgical training. The use of certain technique allows cost to be kept at a minimum. The complications that were encountered in this experience are minor and mainly included superficial wound infection, which could have been avoided by modification in technique.

II106/SOGD

Virtual-Reality Model for Grading Surgical Dexterity in Suture Placement and Customisation of Needle Shape

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Aim: To develop a virtual-reality model to compare dexterity in suture placement in individuals at various levels of surgical skill and analyse the performance with different needle shapes.

Methods: A virtual-reality model consisting of a horizontal membrane, 5 needle shapes and a needle holder was developed. Three standard needle shapes (2/8, 3/8, and 4/8 circles) were used. Two new needles were constructed incorporating a combination of curved and straight segments based on differential curvature of hand motion seen in suture placement. The exercise involved passing the needle through the membrane as in placing sutures using a robotic interface. The needle holder and needle were programmed to be weightless and the membrane was programmed without any mechanical resistance. All movements including physiological tremor from the subject could be faithfully represented. Surgically trained and untrained subjects were used to complete the exercise with normal and 5X magnification on the computer.

Results: Parameters were recorded and dexterity of the subjects in relation to each individual needle shape was analysed. The outcome is presented.

Conclusion: The precision of suture placement varies with the surgeon as well as on the shape of the needle for a given surgical task and indicates a need for needle shape customisation as opposed to usage of fixed radius needles.

II107/SOGD

Attitudes of Obstetricians and Gynaecologists in Singapore on Stem Cell Research and Cloning

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Aim: Stem cell research is controversial and imposes complex challenges for many countries to balance the needs of science in advancing our understanding and treatment of serious diseases with moral, social and ethical concerns. This

survey studied the attitudes of obstetricians and gynaecologists in Singapore on stem cell research and cloning.

Methods: A postal survey of local practising obstetrics and gynaecology (O&G) specialists conducted in December 2001 through the Obstetrical & Gynaecological Society of Singapore.

Results: A total 62 (28.6%) of the 217 O&G specialists surveyed, responded within 3 weeks from the time of posting. The majority were in favour of animal cloning (71.0%), adult stem cell research (88.7%) and embryonic germ stem cell research (from aborted fetuses) (67.7%). Opinions were about equally divided with regards to embryonic stem (ES) cell research (early embryo <14 days) (46.8% in favour) and therapeutic cloning (50.0% in favour). Only a minority was in favour of reproductive cloning (8.1%). Conscientious objectors to participate in treatment to terminate pregnancy under Termination of Pregnancy Act were more conservative as were female O&G specialists. Senior specialists though more liberal in their views on most aspects, were conservative on reproductive cloning. Logistic regression analysis showed conscientious objector status was more influential than sex, seniority or race in determining the view on therapeutic cloning and embryonic stem cell research.

Conclusion: There was strong support for animal cloning and adult stem cell research but support for embryonic stem cell research and therapeutic cloning was equivocal. The majority were against reproductive cloning.

II108/SOGD

Successful Laparoscopic Treatment of an Abdominal Pregnancy in the Broad Ligament

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Aim: Laparoscopic management of tubal ectopic pregnancy has been accepted as the method of choice in most centres. Approximately 1% of ectopic pregnancies are abdominal pregnancies and even rarer are pregnancies in the broad ligament. These are usually managed by laparotomy because of the presentation of acute abdomen or the risk of massive bleeding. We present a case report of laparoscopic treatment of a large 6.5-cm abdominal pregnancy in the broad ligament with focus on the surgical aspects that contributed to its success.

Methods: A 25-year-old lady in good health presented at 10 weeks amenorrhoea for routine antenatal care. An ectopic pregnancy was diagnosed on routine ultrasound. Laparoscopy was undertaken and an ectopic pregnancy in the broad ligament was removed. There were no intra-operative complications and the patient recovered well enough to be discharged on the second post-operative day.

Results and Conclusion: To our knowledge, this case is the first successful laparoscopic management of such a large (6.5-cm) abdominal pregnancy in the broad ligament. Contrary to the views held previously, we believe our case has illustrated that with appropriate patient selection, advancement in laparoscopic setup and skills, a multifaceted anticipatory approach to control bleeding, laparoscopic management of such an abdominal pregnancy is a viable alternative.

II109/SOGD

Endoscopic Assisted "Keyhole" Approach For the Clipping of Basilar Artery Aneurysms

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Aim: Basilar artery aneurysms (BAA) are one of the most challenging lesions to treat in neurosurgery, due to their deep location, inaccessibility, and close proximity to many vital structures. Surgical techniques evolved require extensive skull base exposures and craniotomies for access. This paper seeks to demonstrate the feasibility of a minimally invasive approach.

Methods: All aneurysm patients who presented to the neurosurgery department since 1990 were analysed. Patients who had BAAs were selected for further study. Their case records, imaging studies, clinic records and video recordings

of their surgeries were carefully reviewed and analysed.

Results: 18 patients with BAA were successfully treated by an endoscopic assisted 'keyhole' approach via a 2-3cm supraorbital craniotomy. Their BAAs were well obliterated, and there were no procedure related complications. Because of the minimally invasive approach, their post-surgical recovery was also very rapid.

Conclusion: Endoscopic assisted 'keyhole' surgery is a valuable treatment option for BAAs, with less morbidity than conventional techniques.

II110/SOGD

A National Cytomegalovirus (CMV) Retinitis Screening Programme — Evaluation of Enrollment Criteria

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Aim: To select the minimum subset of criteria for CMV retinitis screening.

Methods: HIV patients with any of the 4 criteria: visual complaints, CD4 <50 cells/microlitre, AIDS-defining illnesses, and CMV elsewhere were enrolled. Combinations of the 4 criteria were analysed.

Results: 14 of 341 screenings were positive. During this period, no CMV retinitis was reported outside this programme. With visual symptoms alone, workload reduced by 93.84%; sensitivity was 21.43%. Excluding the 4th criteria reduced workload by 5.28% but maintained 100% sensitivity.

Conclusion: 78.57% of CMV retinitis patients were asymptomatic. We propose using at least the first 3 criteria (visual complaints, CD4 <50 cells/microlitre and AIDS-defining illnesses) for screening.

II111/SOGD

Cytomegalovirus (CMV) Retinitis in Two Severely Immunocompromised Patients with Systemic Lupus Erythematosus (SLE)

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Aim: To describe 2 cases of CMV retinitis in SLE patients who were heavily immunosuppressed as a result of immunosuppressant therapy.

Methods: Case reports.

Results: The first patient with SLE and active lupus nephritis on mycophenolate mofetil developed right CMV retinitis which responded to weekly intravitreal ganciclovir injections and substitution with systemic prednisolone. The second patient with associated antiphospholipid syndrome (APS) treated with prolonged high-dose (60 mg/day) prednisolone developed CMV retinitis which responded to dose reduction without specific anti-CMV treatment.

Conclusion: Heavily immunosuppressed SLE patients who develop visual symptoms should be evaluated for CMV retinitis. Treatment options include immunotherapy dose reduction and/or specific anti-CMV treatment.

II112/SOGD

Telemedicine Screening for Cytomegalovirus (CMV) Retinitis using Digital Photography

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Aim: To validate a telemedicine screening programme for CMV retinitis.

Methods: HIV patients with any 1 of the following 4 criteria: visual complaints, CD4 count <50 cells/microlitre, AIDS-defining illnesses and CMV elsewhere underwent digital photography using a Zeiss FF450 fundus camera with a Kodak DCS620 digital back. The 9 standard photography fields of 50° each were centred on the macula. Panoramic reconstruction was done and the images were captured in raw tiff format. They were read on a 21-inch Trinitron CRT by a qualified ophthalmologist. This was validated by indirect ophthalmoscopy done by another ophthalmologist.

Results: Of 682 eyes, 10 were unreadable. Of the remaining 672 eyes, 2.53% had CMV retinitis. Photographic screening was 100% sensitive and 99.84% specific. 1 eye with no CMV retinitis was read as suspicious due to an artefact.

Conclusion: Telemedicine screening for CMV retinitis is as sensitive and specific as indirect ophthalmoscopy.

II113/SOGD

Induced Corneal Changes Following Macular Translocation with Punctate Retinotomies and Choriocleral Infolding (Limited Macular Translocation)

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Aim: To report the sequential changes in corneal topography and astigmatism following macular translocation with punctate retinotomies and choriocleral infolding (limited macular translocation).

Methods: A 46-year-old-man who underwent limited macular translocation for idiopathic subfoveal choroidal neovascularisation in the right eye was evaluated by Orbscan (Bausch & Lomb, Rochester, New York, USA) topography and manifest refraction pre-operatively and serially for 1 year post-operatively.

Results: There was an increase in astigmatism corresponding to the area of choriocleral infolding which persisted for 1 year after surgery. Vector analysed astigmatic change showed significant surgically induced astigmatism of 2.18D @ 52.9 degrees, 2.17D @ 57.8 degrees and 2.56D @ 59.1 degrees at 2, 5 and 12 months after surgery.

Conclusion: Corneal changes are evident after limited macular translocation and may remain up to 1 year after the surgery.

II114/SOGD

Effectiveness of Cure of LED Curing Lights at Varying Cavity Depths

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Aim: To compare the effectiveness of cure of 2 LED (light-emitting diodes) lights (Elipar FreeLight [FL], 3M-ESPE; GC e-Light [EL], GC) to conventional (Max [MX] (control), Dentsply-Caulk), high intensity (Elipar TriLight [TL], 3M-ESPE), and very high intensity (Astralis 10 [AS], Ivoclar Vivadent) halogen lights at varying cavity depths. Ten different light curing regimens including pulse (EL1), continuous (FL1, EL2, TL1), turbo (EL3, AS1) and soft-start (FL2, EL4, TL2) modes of various lights were also investigated.

Methods: The effectiveness of cure of the different modes was determined by measuring the top and bottom surface hardness (KHN) of 2, 3 and 4 mm thick composite (Z100, [3M-ESPE]) specimens using a digital microhardness tester (n = 5, load = 500 g; dwell time = 15 seconds). Results were analysed using ANOVA/Scheffe's post-hoc test and Independent Samples t-test (p <0.05).

Results: For all lights, effectiveness of cure was found to decrease with increase cavity depths. The mean hardness ratio for all curing lights at a depth of 2 mm was found to be greater than 0.80 (the accepted minimum standard). At 3 mm, all halogen lights produced hardness ratio greater than 0.80 but some LED light regimens did not; and at a depth of 4 mm, mean hardness ratio observed with all curing lights was found to be less than 0.80. Significant differences in top and bottom KHN values were observed between different curing regimens for the same light, and between LED and halogen lights.

Conclusion: The depth of composite cure with LED curing units was therefore light units and mode dependent.

II115/SOGD

Impact of Clinical Pathway in Subtotal Gastrectomy Cases

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Aim: We evaluated the effect on hospital cost and length of stay with the implementation of clinical care pathway for subtotal gastrectomy patients.

Methods: 9 patients undergoing subtotal gastrectomy were treated according to clinical pathway. The outcomes in terms of total length of stay, postoperative length of stay and cost were compared to 19 pilot patients treated before clinical pathways were implemented. All operations were performed by the same surgeon.

Results: 9 subtotal gastrectomy patients following the clinical pathway had a mean range of 3.22 days to remove the nasogastric tube, 4.22 days to return of bowel sound and 6 days to resume soft diet. Postoperative complications developed in 3 patients (pneumonia, chest atelectasis, wound infection). None were readmitted or underwent re-operation. The mean length of stay and hospital charges decreased from 14.9 days to 10.1 days and \$14,442 to \$12,164 per patient for patients on pathway vs pilot patients. The mean post-operative length of stay decreased from 12.3 days to 8.1 days ($P < 0.033$).

Conclusion: The results of the study showed that treating patients undergoing elective subtotal gastrectomy with clinical care pathway is associated with shorter hospital stay and reduced hospital cost.

II116/SOGD

The Novel Use of Autologous Fats Grafts to Improve Breast Reconstruction – Long-term Results

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Aim: To follow-up and assess the long-term results in patients involved in a pilot study, where fat injections were used to improve breast contour, projection and symmetry after breast reconstruction.

Methods: Autologous fat is harvested via liposuction, using the tumescent technique under local anaesthetic in the outpatient clinic setting. This fat is then injected into areas of defect in the reconstructed breast to improve projection, minor contour defects and symmetry.

Results: The injected fat remained intact at the time of follow-up.

Conclusion: Fat autotransplantation can help improve aesthetic results following breast reconstruction. A permanent aesthetic result is possible in most patients.

II117/SOGD

Clinical Application of a Method of Anthropometric Measurement of the Female Breast

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Aim: The difficult physiognomy of the female breast can make preoperative and postoperative evaluation a challenge in clinical practice. A simple and easily reproducible, yet clinically applicable protocol is required to allow preoperative prediction of the desired breast shape and volume, following breast augmentation, reduction and reconstruction. Such a protocol also has a role in evaluating surgical results. Our unit presents such a protocol for the anthropometric measurement of breasts pre and postoperatively.

Methods: Anthropometric measurements are taken pre- and post-operatively of all women undergoing breast augmentation, reduction and reconstruction in our unit. Measurements of the breast itself, its landmarks and their position from fixed skeletal points are taken. Easily reproducible measurements from the midline are also taken, together with the patient's torso width at the nipple, armpit and intermammary fold.

Results: The most important clinical adaptation of this data was in accurately demonstrating asymmetry of the breasts in patients pre- and post-operatively. The results allowed objective assessment of surgical results following breast reduction and reconstruction, when the pre- and post-operative data were compared.

Conclusion: The data collected via this method allow us to accurately predict breast size post reconstruction following a mastectomy, as well as allow us to plan the appropriate surgery in breast augmentation and reduction, in achieving an aesthetically near-perfect breast.

II118/SOGD

Laparoscopic Intra-gastric Resection of Benign Stromal Tumour—Description of Technique and Review of Literature

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Aim: Our aim was to study the feasibility of a combined laparoscopic-endoscopic 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. A diagnostic gastroscopy showed a 3.8-cm smooth submucosal mass in the lesser curve close to the cardia. An EUS showed it to be well circumscribed and submucosal. A diagnostic laparoscopy was performed by creating an open pneumoperitoneum through the umbilicus. A gastroscope was then inserted and 3 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. The stomach was tested for a leak intraoperatively.

Results: A gastrograffin study was done on the 3rd postoperative day and was found to be normal. The patient made an uneventful recovery and was discharged on the 5th postoperative day. The final histology was leiomyoma.

Conclusion: Our report supports existing literature on the feasibility of a minimally invasive combined technique for removal of gastric stromal tumours. Our technique is also safe for lesions located close to the cardia avoiding the need for any gastric resection.

II119/SOGD

Enhanced Potential of PCL Membrane for Tissue Engineering Periodontal Ligament

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Aim: One way to regenerate the periodontal ligament (PDL) involves the implantation of PDL cells in bioresorbable membranes and scaffolds into defect sites. Our previous study with membrane-scaffold double construct implanted subcutaneously into nude mice demonstrated promising results. In this study, we report 2 methods of culturing PDL *in vitro* as multi-layered cell sheets, and their characterisation on cell-permeable poly-caprolactone (PCL) membranes.

Methods: Cells derived from healthy PDL of human molars were seeded (10,000 cells/cm²) onto 2 sides of membrane, 8 to 10 μ m in thickness, perforations of 100 μ m in diameter. Membranes were then subjected to adherent and suspension culture methods.

Results: Cell attachment was visualised with phalloidin staining. Viability, metabolism and proliferation were monitored with FDA/PI, alamarBlue™ and dsDNA quantitation assays respectively. Protein expression was analysed via Western blotting. Preliminary results showed that cells expressed major ECM proteins such as collagen type I and III, fibronectin, osteonectin and osteopontin. Cell bridging across perforations was observed with the formation of integrated double-sided cell sheets.

Conclusion: This study indicated the potential application of a cell-permeable membrane in combination with cell sheet technology in the tissue-engineering of the PDL *in vitro*.

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NG Chee Kwan	I156/SOGD	S115
NG Ivan	I062/MP	S93
NG Ivan	I168/SOGD	S118
NG Ivan	II054/MP	S147
NG Kar Hui	I076/MP	S96
NGIAM Nicola Siew Pei	I013/MP	S81
OH Meng Choo	I072/MP	S95
OMAR Abdul Razakjr	I010/MP	S81
ONG Clint Ming Lung	II016/MP	S138
ONG Eugene Mun Wai	II096/SOGD	S157
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ONG Hean Yee	I006/MP	S80
ONG Kiat Hoe	I040/MP	S88
ONG Lue Ping	II082/PD	S153
ONG Say How	I011/MP	S81
ONG Wei Chen	I165/SOGD	S117
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OOI Shirley Beng Suat	I030/MP	S85
OTTOSEN Lise Hoej	II037/MP	S143
P Anuradha	II055/MP	S147
PA Ravi	I112/PD	S105
PAN James Chuan-Hsin	II113/SOGD	S160
PILLAI Suresh	II101/SOGD	S158
PNG Constance	I026/MP	S84
POH Kian Keong	II05/MP	S136
POON Lye Yin	I114/PD	S105
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PRAMONO Zacharias A Dwi	II070/MP	S151
PUA Yong Hao	I121/PD	S107
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PUAH Ken Lee	I066/MP	S94
QUAY Irene	I120/PD	S107
RAHMAT Juwita N	II095/PD	S156
RAMLI Aliza	II035/MP	S142
RAO Anil	I218/SOGD	S129
RAO Anil	I226/SOGD	S131
RASUL Golam Haider	I132/SOGD	S110
SAW Tzuen Yih	I134/SOGD	S110
SEAH Irene	I059/MP	S92
SEAH Soek Tian, Angeline	I035/MP	S86
SEE Jee Jian	I130/SOGD	S109
SEE Ju Yaw	I042/MP	S88
SEET Raymond Chee Seong	II034/MP	S142
SENG Boon Kheng		S43
SEOW Shih Wee	I096/MP	S101
SEOW Swee-Chong	II06/MP	S136
SETHU Swaminathan	I204/SOGD	S126
SHAKESPEARE Thomas	I098/MP	S102
SHAKESPEARE Thomas	I099/MP	S102
SHAKESPEARE Thomas	I100/MP	S102
SHAKESPEARE Thomas	II076/MP	S152
SIEW Chee Weng		S69
SIM Kang	I024/MP	S84
SIM Kang	II03/MP	S135

SINGAPOREWALLA Reyaz Moiz	II118/SOGD	S161	VIRYANTI	I093/MP	S100
SIOU Anthony	II108/SOGD	S159	VONG Amy Man In	I116/PD	S106
SITOH Yih Yian	I017/MP	S82			
SNG Jen-Hwei		S53	WAGLE Ajeet	I235/SOGD	S133
SNG Khar Kee, Jeanne	II085/PD	S154	WAGLE Ajeet	I236/SOGD	S134
SO Jimmy Bok Yan	II115/SOGD	S160	WANG Jian Cheng	I039/MP	S87
SOH Mui Siang	II114/SOGD	S160	WANG Lingzhi	I015/MP	S82
SOH Seok Chin	I110/PD	S104	WANG Shih-chang	II086/PD	S154
SOON Gaik Hong	I073/MP	S96	WANG Wen	II02/GP	S135
SOON Winnie, Shok Wen		S26	WANG Xiaoyan	I208/SOGD	S127
SUBRAMANIAM Mythily	I086/MP	S99	WANSaICHEONG Gervais Khin-Lin	I021/MP	S83
			WANSaICHEONG Gervais Khin-Lin	I022/MP	S83
TAGORE Rajat	II038/MP	S143	WANSaICHEONG Gervais Khin-Lin	II08/MP	S136
TAI Dessmon		S34	WEE Agassi Chiahoong	II036/MP	S143
TAN Alvin Chin Kwong	I150/SOGD	S114	WEE Fong Chi		S41
TAN Boon Yeow	II078/MP	S153	WEE Tze Chao	II103/SOGD	S158
TAN Charles	I159/SOGD	S116	WEI Changli		S71
TAN Colin Siang-Hui	I176/SOGD	S120	WEN Bing	I191/SOGD	S123
TAN Colin Siang-Hui	I177/SOGD	S120	WEON Sung Rhan	I094/MP	S101
TAN Colin Siang-Hui	I178/SOGD	S120	WILDER-SMITH Einar	I050/MP	S90
TAN Hao-Yang	II068/MP	S150	WILDER-SMITH Einar	I051/MP	S90
TAN Huay Cheem	I007/MP	S80	WILDER-SMITH Einar	II041/MP	S144
TAN Jackie Yu-Ling	I032/MP	S86	WONG Chek Hooi	II033/MP	S142
TAN James K	I158/SOGD	S116	WONG Hui-Lin	I068/MP	S94
TAN James K	II104/SOGD	S158	WONG Martin Ngie Liong	I070/MP	S95
TAN James Wen Tien	I135/SOGD	S110	WONG Thien Chong	I214/SOGD	S128
TAN Ker Kan	I155/SOGD	S115	WONG Thien Chong, Marcus	I227/SOGD	S131
TAN KH	II107/SOGD	S159	WONG VicPearly	I197/SOGD	S125
TAN Kong Bing	I077/MP	S97	WONG Teck Yee		S75
TAN Kong Bing	I194/SOGD	S124	WOON Yng Yng Bertha	I219/SOGD	S130
TAN Laura, Mei Lian		S39	WOON Yng Yng Bertha	I228/SOGD	S132
TAN Maybelle Phui Shan	I004/MP	S79	WU Qing Hui	I127/PD	S108
TAN Maybelle Phui Shan	II116/SOGD	S161	WU Serene	I203/SOGD	S126
TAN Maybelle Phui Shan	II117/SOGD	S161	WU Vicky Xiaowa	I209/SOGD	S127
TAN Michelle	II089/PD	S155			
TAN Seng Hoe	II025/MP	S140	XU Weixin		S48
TAN Soo Yong	II088/PD	S155			
TAN Tony	I137/SOGD	S111	YAM Andrew	I164/SOGD	S117
TAN Wan Cheng	I045/MP	S89	YAP Adrian	I206/SOGD	S127
TAN Wan Cheng	I048/MP	S90	YAP Elgin	I063/MP	S93
TAN Yvette		S27	YAP Elgin		S63
TANG Johann	I085/MP	S98	YAP Kevin Yi-Lwern	I082/MP	S98
TANG Loon-Ching	II01/GP	S135	YAP Kevin Yi-Lwern	II092/PD	S156
TANG Phua Hwee	I016/MP	S82	YAP Philip Lin Kiat	I036/MP	S87
TANG Phua Hwee	I061/MP	S93	YAP Serene Suen Po	II094/PD	S156
TAY Cheryl Muan Chu	I118/PD	S106	YAP Te-Lu	I080/MP	S97
TEE Lee Huan	I084/MP	S98	YASH PAL Rakhee	I192/SOGD	S123
TEO Swee Guan	I008/MP	S80	YE Lei		S21
TEO Yee Hong	I188/SOGD	S122	YEO Egwin Jun-Chern	I205/SOGD	S126
TEO Yee Hong	I189/SOGD	S123	YEO Leonard	I174/SOGD	S119
TEOH Stephen Charn Beng	I187/SOGD	S122	YEO Leonard	I175/SOGD	S120
THAM Kum Ying	I029/MP	S85	YEOH Lee Ying	I047/MP	S89
THAM Lai San		S46	YEOH Lee Ying	II044/MP	S145
THONG Bernard	I091/MP	S100	YIM Heng Boon	I033/MP	S86
THONG Bernard	II072/MP	S151	YIP Chee Chew	I181/SOGD	S121
THONG Bernard	II073/MP	S152	YIP Leonard	I064/MP	S94
THONG Bernard	II074/MP	S152	YONG Ren	II022/MP	S139
TIONG Ho Yee	I224/SOGD	S131	YONG Vernon	I145/SOGD	S113
TIONG Ho Yee	I233/SOGD	S133	YUAN PEI	I166/SOGD	S118
TIONG Ho Yee	I234/SOGD	S133	YUEN Heng Wai	I143/SOGD	S112
TOH Lydia	II093/PD	S156			
TOH Teck Hock	I018/MP	S83	ZAINUDIN Sueziani	I055/MP	S91
TONG Cherie	II087/PD	S155	ZHANG Dao-Hai		S77
			ZHANG Xiao Jian	I092/MP	S100
VELUCHAMY AMUTHA Barathi	I095/MP	S101	ZHAO Feng	II09/MP	S136
VENKATARAMAN Ashok	I149/SOGD	S114	ZHOU Lei	I103/MP	S103
VERMA Swapna	I025/MP	S84	ZOU Xiaohui	I201/SOGD	S125
VERMA Swapna	I027/MP	S85			