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*"Our brains have been designed to blur the line between self and other.
It is an ancient neural circuitry that marks
every mammal, from mouse to elephant."*

Frans de Waal, (1948 –)
Dutch primatologist and ethologist

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Blood Stem Cell Donation: A Model for Worldwide Cooperation in Transplantation

William YK Hwang,^{1,2}*MBBS, FAMS, FRCP*, Lydia M Foeken,^{3,4}*BSc*

Haematopoietic stem cell transplantation (HSCT) has been carried out for more than 50 years in over 50 countries.¹ Over a million patients have undergone this procedure, giving hope and a new lease of life to many patients with otherwise incurable or high-risk blood-related cancers and other diseases.² The procedure involves taking good blood stem cells from an individual and giving them to a patient who has just received a preparative regimen of high dose chemotherapy and/or radiation. These blood stem cells may be autologous if they are derived from the patient himself or allogeneic if obtained from another individual. While a human leukocyte antigen (HLA) matched sibling is the first choice as a donor of these blood stem cells, only a quarter of patients have a suitable and matched sibling donor, and an unrelated or mismatched donor may need to be used.

While many of the initial transplants were carried out using cells donated from the bone marrow, the last two decades have seen an increase in the use of blood stem cells obtained from the peripheral blood after these cells are mobilised into the peripheral blood with the use of granulocyte colony stimulating factor (G-CSF).³ The use of umbilical cord blood as a source of cells for haematopoietic transplants has helped increase donor availability, especially since these cells appear to have a greater ability in tolerating mismatches during transplantation⁴ and consequently, it was even suggested that fewer donors of cord blood are required to establish a significant pool of donors for transplantation for patients in need.⁵

In the last two years, we have seen the passing of two individuals who laid the pioneering groundwork for the worldwide practice of bone marrow transplantation—Dr E. Donnall Thomas and Dr John Goldman. Professor Thomas was awarded the Nobel Prize for being the founding father of the science of bone marrow transplantation and a true pioneer who founded a completely new field that has given hope to many patients. The world mourned his passing in 2012, which was also the year in which the one-millionth

HSCT was performed.⁶ John Goldman was widely respected in the field of haematology and he was one of the pioneers who laid the foundation stones for the field of unrelated donor marrow transplantation.⁷ Together with Professor Jon Van Rood, who made crucial discoveries in the role of HLA and laid the groundwork for the tissue typing that would make blood stem cell transplants possible, Professor Goldman set in motion the first steps in the creation of the World Marrow Donor Association (WMDA), with Donnall Thomas as its first President.

The WMDA is the de facto global organisation that many look to for standards and answers pertaining to unrelated donor blood stem cell donation. It also coordinates the activities of blood stem cell donor and cord blood registries around the world.⁸ The WMDA plans and implements standards for blood stem cell registries and develops recommendations that help to ensure the safety of donors and patients around the world.⁹ The Serious (Product) Events and Adverse Reactions (S(P)EAR) committee of the WMDA has contributed to the monitoring and enhancing the safety of our donors and recently launched a first-of-its-kind online reporting system for global vigilance and surveillance in unrelated donors who have donated blood stem cells for transplantation.¹⁰

The WMDA turned 25 years old in the year 2013 and, thanks to the groundwork laid down through the last two decades of hard work, it is now meeting its mission to “foster international collaboration to facilitate the exchange of high quality blood stem cells for clinical transplantation worldwide and to promote the interests of donors”.^{11,12} The WMDA network now represents more than 73 donor registries, 160 cord blood banks, and 350 donor centres, which have more than 24 million donors listed in 55 different countries. Thanks to the concerted efforts of cord blood banks and blood stem cell registries around the world, transplant centres now report that there is now greater ease in finding a suitable unrelated donor or cord blood unit than

¹Department of Haematology, Singapore General Hospital, Singapore

²Cancer and Stem Cell Biology Program, Duke-NUS Graduate Medical School, Singapore

³World Marrow Donor Association, Netherlands

⁴Europdonor, Netherlands

Address for Correspondence: Dr William Hwang, Department of Haematology, Singapore General Hospital, Outram Road, Singapore 169608.

Email: william.hwang.y.k@sgh.com.sg

a decade ago. Our patients now have good chances in finding a donor from another part of the world when one cannot be found in their own country. Our donors also have the exciting prospect of being able to save the life of another person in another corner of the globe. In fact, there are now around 33 stem cell products being transported every day across the world to facilitate transplants in another country. The extent, regularity, robustness and high standards of the over 12,000 haematopoietic stem cell donor products being exchanged across borders every year to save the lives of patients in need is truly staggering. Blood stem cell donation has indeed become a model for worldwide cooperation in transplantation.

HSCT procedures are now safer as well as more effective, and the medical community has come to accept HSCT as an established form of therapy. Many physicians now recognise that HSCT does not necessarily compete with newer therapies but serves as an important adjunct to newer drugs. In recent years, work done by researchers around the world has led to tremendous advances in HSCT. Rigorous clinical studies coordinated by worldwide networks like the Center for International Blood and Marrow Transplantation Research (CIBMTR)¹³ and the advent of safer and more effective new drugs have now made transplants much safer and it is now possible to transplant older and sicker patients by reducing the risk of infectious and other complications. The advent of new technologies involving the expansion of haematopoietic stem cells^{14,15} as well as the generation of cells with potential anti-leukaemia effects^{16,17} have led to a whole new realm of possibilities for HSCT.

Through the coordinated efforts of many countries in improving the efficacy and safety of blood stem cell transplants, the future of HSCT is bright indeed.

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Creation and Validation of the Singapore Birth Nomograms for Birth Weight, Length and Head Circumference Based on a 12-year Birth Cohort

Woei Bing Poon,^{1,2,4} MBBS, MRCPCH, FAMS, Stephanie MC Fook-Chong,³ BSc, MSc, Grace YL Ler,⁴, Zhi Wen Loh,⁴, Cheo Lian Yeo,^{1,2,4} MBBS, MMed(Paed), FAMS

Abstract

Introduction: Both gestation and birth weight have significant impact on mortality and morbidity in newborn infants. Nomograms at birth allow classification of infants into small for gestational age (SGA) and large for gestational age (LGA) categories, for risk stratification and more intensive monitoring. To date, the growth charts for preterm newborn infants in Singapore are based on the Fenton growth charts, which are constructed based on combining data from various Western growth cohorts. Hence, we aim to create Singapore nomograms for birth weight, length and head circumference at birth, which would reflect the norms and challenges faced by local infants. **Materials and Methods:** Growth parameters of all babies born or admitted to our unit from 2001 to 2012 were retrieved. Following exclusion of outliers, nomograms for 3 percentiles of 10th, 50th, and 90th were generated for the gestational age (GA) ranges of 25 to 42 weeks using quantile regression (QR) combined with the use of restricted cubic splines. Various polynomial models (second to third degrees) were investigated for suitability of fit. The optimum QR model was found to be a third degree polynomial with a single knotted cubic spline in the mid-point of the GA range, at 33.5 weeks. Check for goodness of fit was done by visual inspection first. Next, check was performed to ensure the correct proportion: 10% of all cases fall above the upper 90th percentile and 10% fall below the lower 10th percentile. Furthermore, an alternative formula-based method of nomogram construction, using mean, standard deviation (SD) and assumption of normality at each gestational age, was used for counterchecking. **Results:** A total of 13,403 newborns were included in the analysis. The new infant-foetal growth charts with respect to birth weight, heel-crown length and occipitofrontal circumference from 25 to 42 weeks gestations with the 10th, 50th and 90th were presented. **Conclusion:** Nomograms for birth weight, length and head circumference at birth had significant impact on neonatal practice and validation of the Singapore birth nomograms against Fenton growth charts showed better sensitivity and comparable specificity, positive and negative predictive values.

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Key words: Fenton, Percentile, Prematurity, Preterm growth charts, Very low birth weight

¹Department of Neonatal and Developmental Medicine, Singapore General Hospital, Singapore

²Duke-NUS School of Medicine, Singapore

³Division of Research, Singapore General Hospital, Singapore

⁴Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Address for Correspondence: Dr Poon Woei Bing, Department of Neonatal and Developmental Medicine, Singapore General Hospital, Outram Road, Singapore 169608.

Email: poon.woei.bing@sgh.com.sg

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Woei Bing Poon,^{1,2,4} MBBS, MRCPCH, FAMS, Stephanie MC Fook-Chong,³ BSc, MSc, Grace YL Ler,⁴, Zhi Wen Loh,⁴, Cheo Lian Yeo,^{1,2,4} MBBS, MMed(Paed), FAMS

Abstract

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Introduction

Both gestation and birth weight have significant impact on mortality and morbidity in newborn infants.^{1,2} Nomograms at birth allow classification of infants into small for gestational age (SGA) and large for gestational age (LGA) categories, based on <10th percentile and >90th percentile birth weights respectively. These categorisations allow for risk stratification and more intensive monitoring.

A recent review stated that uncertainty existed regarding the most suitable curves for monitoring the growth of preterm

infants. While intrauterine growth rate appeared to be the ideal growth that needs to be attained by the preterm infants, there were considerable problems with its accuracy and it may not be feasible to attain such growth trajectories, given the limitations set by the morbidities of prematurity.³ Bertino et al also stated in a review on neonatal charts that localised or national growth charts were preferred by clinicians, and that these should be descriptive “how growth actually is” serving as a reference, and should be updated every 5 to 10 years or at the most 15 to 20 years.⁴ However, the last

¹Department of Neonatal and Developmental Medicine, Singapore General Hospital, Singapore

²Duke-NUS School of Medicine, Singapore

³Division of Research, Singapore General Hospital, Singapore

⁴Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Address for Correspondence: Dr Poon Woei Bing, Department of Neonatal and Developmental Medicine, Singapore General Hospital, Outram Road, Singapore 169608.
Email: poon.woei.bing@sgh.com.sg

published growth charts for Singapore was in 1972 and in 1994 for Malaysia.^{5,6}

Currently, most neonatal centres use variations of charts developed by Babson and Benda, Lubchenco or Usher.³ The Babson and Benda's chart, which was 4 decades old, had been updated by Fenton, using recent data from larger samples of preterm infants, making it useful for monitoring growth of infants in the preterm period.^{7,8} Once a corrected age of 40 weeks is reached, the appropriate paediatric growth curves can be used to monitor their ongoing growth.

To date, the growth charts for newborn infants in Singapore are based on the Fenton growth charts, which are constructed based on combining data from various Western growth cohorts from the 1990s.

Our study aimed to create updated Singapore birth nomograms for birth weight, length and head circumference at birth, which would reflect the norms and challenges faced locally.

Materials and Methods

Growth parameters of all babies born or admitted to our unit from 2001 to 2012 were included. Babies with major congenital malformations were excluded. Gestational age (GA) by completed weeks was determined by early ultrasound dating or by last menstrual period. If the gestation was less than 37 weeks, it was counterchecked with the Dubowitz or new Ballard scoring post-delivery. Where Dubowitz or Ballard scoring differed by more than 2 weeks from the last menstrual period and in the absence of early dating scans, the Dubowitz- or Ballard-scored GA was taken.

All parameters were measured immediately after birth or at the latest, within 24 hours after birth. Birth weight was taken using digital weighing scales accurate to the nearest gram, after the baby was dried. Head circumference was taken by using the occipitofrontal circumference with a measuring tape. Length was taken with a stadiometer, as part of the weighing scale, using heel-crown length. Measurements were rounded to the nearest centimetre.

Possible erroneous data entry, measurement or recording of birth weight, head circumference and crown-heel length were identified in 2 ways. First, visual inspection of box-and-whisker plots of each live birth measurement by GA identified obvious implausible data points. Next, the statistical method of Tukey⁹ was used to discard outliers, because the large volume of birth data made it impossible to trace every single suspicious data from the visual examination of box-and-whisker plot. Tukey's method consisted of computing the 25th and 75th percentiles for each GA to get the interquartile range (IQR), IQR being 75th percentile to 25th percentile. Within each GA, data points higher than 1.5 multiples of IQR from the 75th percentile

or lower than 1.5 multiples of IQR from 25th percentile were discarded and not used in the construction of birth nomograms.

Following exclusion of these outliers, nomograms for 3 percentiles of 10th, 50th, and 90th were generated for the GA range of 25 to 42 weeks using quantile regression (QR) combined with the use of cubic splines¹⁰ for each type of measurement. Various polynomial models (second to third degrees) were investigated for suitability of fit to the data. The optimum QR model for the 3 types of live-birth measurements was found to be a third degree polynomial in terms of GA (i.e. a model with linear, quadratic and cubic terms of gestational age) with a single knotted cubic spline in the mid-point of the GA range, at 33.5 weeks. Such a knot was chosen to allow for some change in growth curve direction halfway through the GA range that was observed from the empirical data plotted on a graph.

Check for goodness of fit model was done by visual inspection of the nomograms with the empirical data superimposed on the nomograms. Next, check was performed that approximately the correct proportion of 10% of all cases fall above the upper 90th percentile and 10% of all cases fall below the lower 10th percentile. Furthermore, an alternative simple method of nomograms construction was used for counter-checking, not based on modeling technique, but by formula based on mean and standard deviation (SD) and assumption of normality at each integer GA. For example, the 10th and 90th percentile at a particular GA was calculated respectively as mean — $Z_{0.90} \times \text{SD}$, mean + $Z_{0.90} \times \text{SD}$, where mean and SD are the values at corresponding GA, $Z_{0.90}$ is the z-score value corresponding to an area of 0.9 under the standard normal curve to the left of that z-score ($Z_{0.90} = 1.28$). To obtain any particular centile curve by this method, the centile points for the GAs were connected smoothly by the use of third degree polynomial functions using Excel graphing capabilities. A visual check was then made of how closely the nomograms constructed by QR and simplistic formula based on mean and SD at each GA coincide.

Comparison between the Fenton growth charts and Singapore birth nomograms were performed using SGA clinical outcomes to obtain sensitivity, specificity, positive and negative predictive values. SGA morbidities were defined as one or all of the following: perinatal depression, meconium aspiration, pulmonary haemorrhage, persistent pulmonary hypertension of newborn, hypotension, hypoglycaemia, hypocalcaemia, hypothermia, polycythaemia, neutropenia, thrombocytopenia and renal insufficiency.¹¹ Perinatal depression was defined as Apgar scores at 5 minutes of 5 and below. Persistent pulmonary hypertension of newborn (PPHN) was diagnosed by echocardiography performed by paediatric cardiologists. 2D echocardiography was

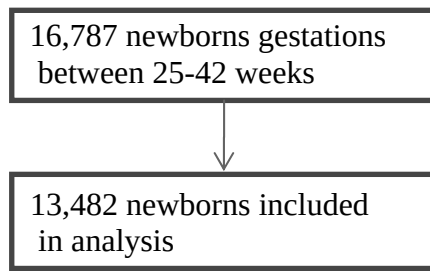


Fig. 1. The dataset was cleaned up with outliers at each gestation group removed by Tukey's method (more than 1.5 boxlength away from 25th or 75th percentile) for birth weight, length and head circumference. Outliers were removed for birth weight, length and head circumference numbered 322, 271 and 580 respectively. Head circumference measurement was missing for 2530 patients before clean-up. The number of missing data for birth weight was 1 and for length it was 72. As we wanted a clean-up dataset with complete data and equal final samples for all 3 measurements, this resulted in 3300 newborns being excluded.

Table 1. Baseline Characteristics

Characteristics	Frequency (%)
25	27 (0.20)
26	34 (0.25)
27	58 (0.43)
28	71 (0.53)
29	86 (0.64)
30	101 (0.75)
31	100 (0.74)
32	144 (1.07)
33	188 (1.39)
34	283 (2.10)
35	352 (2.61)
36	833 (6.18)
37	1680 (12.46)
38	3234 (23.99)
39	3414 (25.32)
40	2366 (17.55)
41	456 (3.38)
42	55 (0.41)
Gender	
Male	6973 (51.72)
Female	6509 (48.28)
Ethnic Group	
Chinese	6055 (44.97)
Malays	5189 (38.53)
Indians	1309 (9.72)
Others	913 (6.78)
Multiple Births	
Singletons	12,909 (95.75)
Twins	518 (3.84)
Triplets	55 (0.41)

not performed for all babies, except only when clinical suspicion of PPHN existed. Hypotension was defined as mean arterial blood pressure less than GA. Hypothermia was defined as axillary temperature less than 36.5 °C, hypoglycemia as plasma glucose level of less than or equal to 2.5 mmol/L and hypocalcemia as plasma total calcium level <2.0 mmol/L or ionic calcium level <1.0 mmol/L. In addition, renal insufficiency was assessed clinically by oliguria and/or if the urea, creatinine tests performed were abnormal. All premature babies defined as less than 37 weeks gestation, whether SGA or non-SGA, would undergo the same clinical and blood tests monitoring in terms of full blood count, serum calcium and blood sugar monitoring regime for the first 24 hours. In term babies, only SGA babies who were low birth weight (less than 2500 g) would have the blood tests monitoring performed, although clinical monitoring were similar.

Results

Figure 1 shows the flowchart for patient inclusion, while Table 1 shows the baseline characteristics of infants included in the analysis. Tables 2, 3 and 4 shows the 10th%, 50th% and 90th% values for the birth weight, head circumference and length respectively. Figures 2, 3 and 4 are diagrammatic representations of the 10th%, 50th% and 90th% values for the birth weight, head circumference and length respectively.

Clinical Validation and Comparisons Between the Fenton Growth Charts and Singapore Birth Nomograms Using SGA Morbidities

Tables 5 and 6 compared the use of the Singapore birth nomogram against the Fenton growth chart, as screening tools for SGA morbidities, when validated against the birth cohort from 2001 to 2012.

Further counter-checking was performed by performing chi-square test for mortality against gestation and against weight percentiles for GA. Statistical significance was found in mortality against gestation ($P < 0.001$) [n (%) for mortality in gestation age groups ≤ 37 weeks, 38-41 weeks, ≥ 42 weeks were 18 (0.46%) versus 0 (0%) versus 0 (0%) respectively], while no statistical significance was found against weight percentiles for GA ($P = 0.614$) [n (%) for mortality in SGA, AGA and LGA were 3 (0.22%) versus 13 (0.12%) versus 2 (0.15%)].

Discussion

In the 1960s, Battaglia et al¹² published a study on birth weights against gestational age from John Hopkins Hospital, while Lubchenco et al¹³ published birth weight against GA distribution from Colorado involving both inborn and

Table 2. Birth Weight Data (in Grams) Used for Constructing Singapore Birth Nomogram Using QR

GA	Mean	SD	Frequency	QR 10 th %	QR 50 th %	QR 90 th %
25	719.6	91.67	27	602.2	775.0	991.3
26	880.9	166.84	34	620.0	845.0	1032.1
27	972.5	164.47	58	671.6	942.3	1130.0
28	1006.3	252.50	71	755.0	1064.6	1276.4
29	1206.5	215.20	86	867.4	1209.7	1462.6
30	1365.2	228.32	101	1005.9	1375.4	1680.0
31	1548.9	284.46	100	1167.7	1559.6	1920.1
32	1734.3	377.91	144	1350.1	1760.0	2174.2
33	1950.4	350.26	188	1550.2	1974.5	2433.8
34	2236.5	436.66	283	1765.0	2200.6	2690.2
35	2453.4	338.52	352	1987.5	2431.7	2935.9
36	2696.7	402.33	833	2206.7	2656.9	3164.7
37	2893.2	352.63	1680	2441.3	2865.0	3370.0
38	3065.9	387.08	3234	2590.0	3045.0	3545.6
39	3187.0	381.88	3414	2731.7	3185.7	3685.0
40	3297.0	363.82	2366	2825.0	3276.1	3781.9
41	3347.6	380.37	456	2858.8	3305.0	3830.0
42	3384.4	518.86	55	2821.8	3261.3	3822.8

GA: Gestational age; QR: Quantile regression; SD: Standard deviation

Table 3. Head Circumference Data (in Centimetres) Used for Constructing Singapore Birth Nomogram Using QR

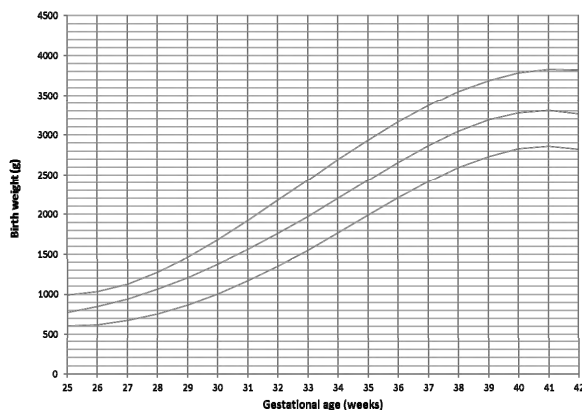
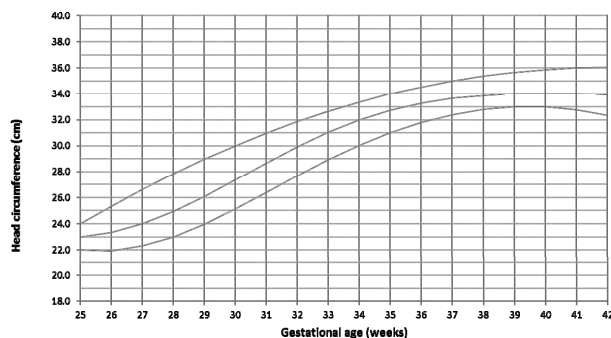
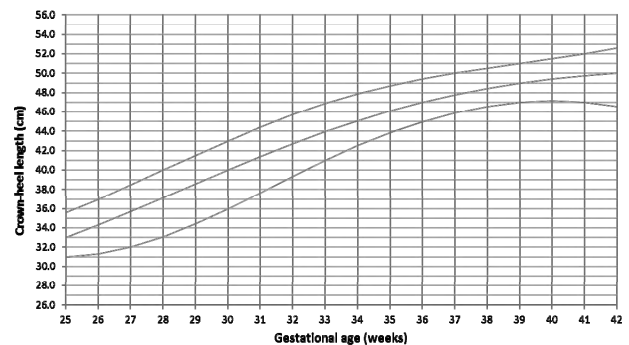
GA	Mean	SD	Frequency	QR 10 th %	QR 50 th %	QR 90 th %
25	22.4	0.84	27	22.0	23.0	24.0
26	24.1	1.52	34	21.9	23.3	25.3
27	24.8	1.36	58	22.3	24.0	26.6
28	25.0	1.85	71	23.0	24.9	27.8
29	26.6	1.50	86	24.0	26.1	29.0
30	27.6	1.62	101	25.1	27.3	30.0
31	28.7	1.47	100	26.4	28.6	31.0
32	29.6	1.83	144	27.7	29.9	31.9
33	30.7	1.63	188	28.9	31.0	32.7
34	31.8	1.53	283	30.0	32.0	33.4
35	32.5	0.95	352	31.0	32.7	34.0
36	33.2	1.17	833	31.8	33.3	34.5
37	33.6	0.90	1680	32.4	33.6	35.0
38	33.9	1.14	3234	32.8	33.8	35.4
39	34.0	1.08	3414	33.0	34.0	35.7
40	34.3	0.94	2366	33.0	34.0	35.9
41	34.4	0.95	456	32.8	34.0	36.0
42	34.6	0.94	55	32.3	33.9	36.0

GA: Gestational age; QR: Quantile regression; SD: Standard deviation

Table 4. Length Data (in Centimetres) Used For Constructing Singapore Birth Nomogram by QR

GA	Mean	SD	Frequency	QR 10 th %	QR 50 th %	QR 90 th %
25	32.6	1.25	27	31.0	33.0	35.6
26	34.8	2.56	34	31.3	34.3	37.0
27	35.8	2.27	58	32.0	35.7	38.5
28	36.4	3.18	71	33.1	37.1	40.0
29	38.4	2.58	86	34.5	38.6	41.5
30	39.9	2.17	101	36.0	40.0	43.0
31	41.2	2.62	100	37.7	41.4	44.4
32	42.3	2.73	144	39.4	42.7	45.7
33	43.4	2.30	188	41.0	44.0	46.9
34	44.9	2.45	283	42.5	45.1	47.9
35	46.1	1.74	352	43.8	46.1	48.7
36	47.1	1.77	833	45.0	47.0	49.4
37	47.9	1.68	1680	45.9	47.8	50.0
38	48.5	1.70	3234	46.6	48.4	50.5
39	49.1	1.61	3414	47.0	49.0	51.0
40	49.5	1.57	2366	47.1	49.4	51.5
41	49.7	1.60	456	47.0	49.8	52.0
42	49.7	2.00	55	46.5	50.0	52.6

GA: Gestational age; QR: Quantile regression; SD: Standard deviation

Fig. 2. Chart showing Singapore birth nomogram with 10th, 50th and 90th percentile for birth weight by gestational age.Fig. 3. Chart showing Singapore birth nomogram with 10th, 50th and 90th percentile for head circumference by gestational age.Fig. 4. Chart showing Singapore birth nomogram with 10th, 50th and 90th percentile for crown-heel length by gestational age.

outborn infants. The two studies, published within a few years of each other, showed remarkable agreement in the 10th percentile of the birth weight against GA plot, but differed markedly in the 90th percentile distribution.¹⁴ Since then, birth nomograms for preterms and terms had significant impact on neonatal practice, particularly if customised for the individual population.¹⁵

A recent review of mortality rates conducted under the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network employed the approach of these original studies, using birth weight and GA data to construct mortality rates.¹⁶ Unfortunately, the article “did not present the data in a useable format that related mortality to both gestational age and birth weight.

Table 5. SGA Morbidities Using Singapore Birth Nomograms

GA	SGA	None-SGA	Percentage SGA	True Positive	False Positive	True Negative	False Negative	Sensitivity	Specificity
25	6	28	17.65%	6	0	2	26	18.75%	100.00%
26	2	36	5.26%	2	0	10	26	7.14%	100.00%
27	4	58	6.45%	4	0	21	37	9.76%	100.00%
28	10	73	12.05%	9	1	26	47	16.07%	96.30%
29	8	84	8.70%	7	1	43	41	14.58%	97.73%
30	13	99	11.61%	11	2	59	40	21.57%	96.72%
31	9	99	8.33%	6	3	68	31	16.22%	95.77%
32	23	131	14.94%	10	13	82	49	16.95%	86.32%
33	27	178	13.17%	10	17	111	67	12.99%	86.72%
34	44	261	14.43%	15	29	180	81	15.63%	86.12%
35	44	394	10.05%	21	23	296	98	17.65%	92.79%
36	117	804	12.70%	37	80	629	175	17.45%	88.72%
37	243	1957	11.05%	65	178	1715	242	21.17%	90.60%
38	356	3613	8.97%	62	294	3284	329	15.86%	91.78%
39	429	3895	9.92%	53	376	3637	258	17.04%	90.63%
40	320	2724	10.51%	46	274	2515	209	18.04%	90.18%
41	64	553	10.37%	8	56	511	42	16.00%	90.12%
42	10	71	12.35%	1	9	65	6	14.29%	87.84%
Total	1729	15058	10.30%	373	1356	13254	1804	17.13%	90.72%

GA: Gestational age; SGA: Small for gestational age

Table 6. SGA Morbidities Using Fenton Growth Chart

GA	SGA	None-SGA	Percentage SGA	True Positive	False Positive	True Negative	False Negative	Sensitivity	Specificity
25	3	31	8.82%	3	0	2	29	9.38%	100.00%
26	5	33	13.16%	5	0	10	23	17.86%	100.00%
27	4	58	6.45%	4	0	21	37	9.76%	100.00%
28	18	65	21.69%	14	4	23	42	25.00%	85.19%
29	9	83	9.78%	8	1	43	40	16.67%	97.73%
30	21	91	18.75%	15	6	55	36	29.41%	90.16%
31	10	98	9.26%	7	3	68	30	18.92%	95.77%
32	23	131	14.94%	10	13	82	49	16.95%	86.32%
33	23	182	11.22%	8	15	113	69	10.39%	88.28%
34	32	273	10.49%	9	23	186	87	9.38%	89.00%
35	30	408	6.85%	18	12	307	101	15.13%	96.24%
36	69	852	7.49%	25	44	665	187	11.79%	93.79%
37	117	2083	5.32%	40	77	1816	267	13.03%	95.93%
38	208	3761	5.24%	44	164	3414	347	11.25%	95.42%
39	318	4006	7.35%	44	274	3739	267	14.15%	93.17%
40	408	2636	13.40%	54	354	2435	201	21.18%	87.31%
41	144	473	23.34%	12	132	435	38	24.00%	76.72%
42	32	49	39.51%	3	29	45	4	42.86%	60.81%
Total	1474	15313	8.78%	323	1151	13459	1854	14.84%	92.12%

GA: Gestational age; SGA: Small for gestational age

However, the only coloured graph in the study suggested no difference for mortality at gestational ages older than 29 weeks whether the infants were LGA or SGA. There was criticism that comparisons were weakened in terms of clinical usefulness by the inclusion of gender differences as well.”¹⁴

Obstetric research pointed out that foetal growth curves constructed from ultrasonographic estimation of fetal size in normal pregnancies defined greater fetal weights at any given GA than the curves using birth weight data. In addition, it is unclear whether calculating an estimated fetal weight from population-based data of multiple fetal measurements is an improvement over using those measurements themselves to detect undersized infants.¹⁷ Ultrasound estimation of birth weights remain fraught with inaccuracies for various reasons.¹⁸

Each nation should construct its own birth nomograms, with their own outcomes based on gestation and SGA versus non-SGA subgroups. This can act as a barometer for their obstetric and neonatal practices, assist in prognostication and counseling of parents and finally, serve as a platform to further improve medical practice and outcomes. In this aspect, the United States was first off, having already published their national growth chart based on their multi-ethnic birth cohort.¹⁶ We believe our study contributed to the literature on Asian ethnicity growth for preterm babies and for Singapore, and at the minimum, provided data for future meta-analyses on growth of preterm babies in Asia.

Visual inspection of the Fenton growth charts and Singapore birth nomograms superimposed together (Figs. 5, 6 and 7) reveal that the Fenton growth chart tends to increase in a linear fashion as it goes into the term and post-term gestations. In contrast, the Singapore birth nomograms show a more sigmoidal S-shaped curve. We believe that the latter corroborates with our clinical experience. Newborns do not grow linearly indefinitely while gestation increases; this growth is expected to tail off as the baby reaches term or post-term. This difference between the charts could be due to the artificial amalgamation of different growth cohorts, preterm versus term, in the making of the Fenton growth charts.

In addition to risk stratification, these growth charts were also important for the monitoring of post-natal growth for premature babies. These infants were at risk for poor growth, whether in the hospital or after discharge. They must be closely monitored and may require interventions to promote better growth.

To date, no studies have been performed to validate how the Fenton growth charts performed in a cohort of real patients, in terms of risk stratification. When we validated the Fenton growth charts on our study population, we found that they had high negative predictive values. This

corroborated with actual clinical practice as the growth charts were used as the first layer of screening in SGA babies, who were then further screened clinically, as well as with blood investigations such as full blood count, blood sugar monitoring and electrolyte checks.

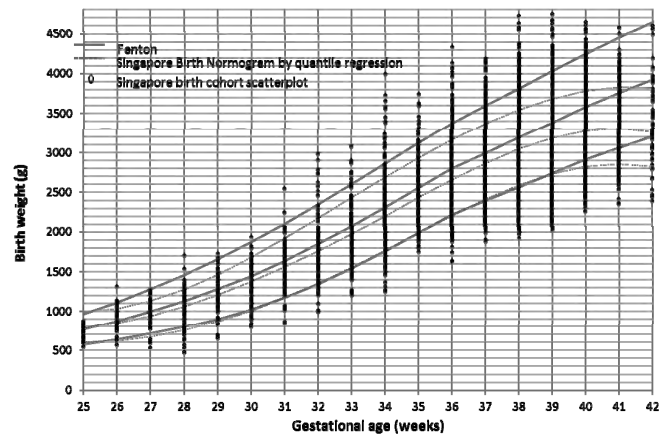


Fig. 5. Chart showing the 10th, 50th and 90th percentiles for birth weight by gestational age.

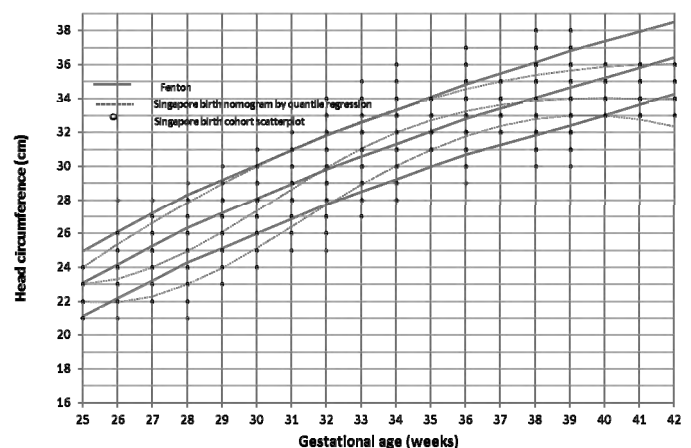


Fig. 6. Chart showing the 10th, 50th and 90th percentiles for head circumference by gestational age.

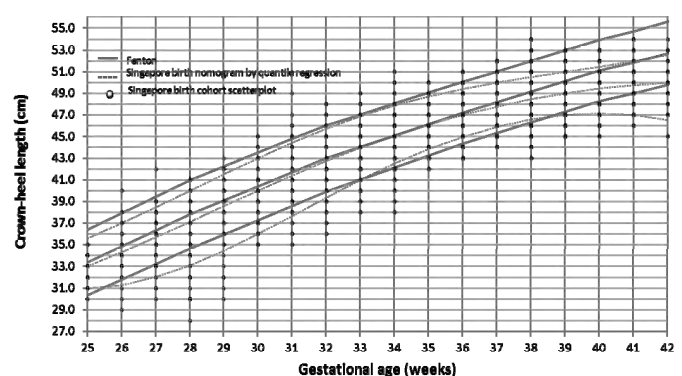


Fig. 7. Chart showing the 10th, 50th and 90th percentiles for crown-heel length by gestational age.

Table 7 summarises the results for Tables 5 and 6 for SGA morbidities screening. Our comparison showed that the Singapore birth nomograms' positive and negative predictive values, as well as specificity, were comparable to the Fenton growth charts. In addition, it had higher sensitivity. We believe this was an important additional advantage as a first line screening tool.

Table 7. PPV, NPV, Sensitivity and Specificity for the Fenton Growth Charts and Singapore Birth Nomograms

Characteristics	Fenton Growth Charts	Singapore Birth Nomograms
PPV	21.91%	21.57%
NPV	87.89%	88.02%
Sensitivity	14.84%	17.13%
Specificity	92.12%	90.72%

NPV: Negative predictive value; PPV: Positive predictive value

Limitations and Future Research

Our cohort may not be representative of the national growth cohort as our centre is only 1 of 3 public tertiary neonatal units, and a substantial proportion of births occur in private centres. However, we believe we have sufficient numbers accumulated over the years to be representative, particularly in the near term and term gestations. In particular, we systematically collected both growth parameters and outcome data prospectively for all babies during admission.

There was over-representation of Malays in our cohort, compared to the ethnic make-up of Singapore. However, Malays had higher fertility rates during the study period compared to other ethnic groups. Malay fertility rate ranged from 1.64 to 2.54 compared to overall population total fertility rate of 1.2 to 1.6. This meant that Malay infants would naturally be over-represented in any local natural birth cohort, as opposed to the general population ethnic make-up. Our construction of separate nomograms for each race also did not show any racial differences.

Head circumference from 39 weeks onwards increased only marginally, for the 50th percentile and 90th percentile curves. We believe that in the future when we have more data at GA 41 and 42 weeks, we will have a more accurate picture of the nomograms at the tail ends of the curves for head circumference. Interestingly, in the Malaysian curves for head circumference by Boo et al,⁶ a similar finding of no more than minimal increase was found at the tail ends too.

There was a possibility of inaccuracies in data entry, although data accuracy was counter-checked during data entry. Hence, we employed various statistical means to remove outliers to improve accuracy.

Further research should include a future prospective study to see how well the Singapore birth nomograms fare in picking up both SGA and LGA morbidities in the various gestations. In addition, prospective multi-centre studies could hopefully be performed in future to construct nomograms and see whether and how much these will defer from these currently constructed nomograms. Hopefully, there would also be further research around the world, and particularly in Asia, to obtain norms at birth for different gestations for different nations and ethnic groups.

Conclusion

Not only were the Singapore birth nomograms based on local birth cohort of Asian ethnicity and more recent births, there was also no artificial amalgamation of different cohorts across gestations, unlike the Fenton growth charts. Validation of the Singapore birth nomograms against Fenton growth charts showed better sensitivity and comparable specificity, positive and negative predictive values.

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Osteogenic Sarcoma in Children and Young Adults

LeLe Aung,¹MD, MPH, Aung Soe Tin,²MBBS, MPH, Thuan Chong Quah,³MBBS, MMed, Robert W Pho,⁴FRCS, MD

Abstract

Introduction: More than 80% of children with osteogenic sarcoma (OS) relapse and 35% to 40% of them die within the first 2 years after diagnosis due to relapse. We investigated the incidence, treatment modalities used and the outcome of patients with OS treated in Singapore. **Materials and Methods:** Patients with OS treated in Department of Paediatrics KK Women's and Children's Hospital (KKH) and National University Hospital (NUH) between January 1994 and June 2011 were reviewed. Chemotherapy was as per the European Osteosarcoma Intergroup (EOI) and as per the Memorial Sloan-Kettering Cancer Centre's (MSKCC) T12 protocols. Overall and event-free (EFS) 5-year survivals were calculated using Kaplan-Meier analysis and Cox proportional hazards regression analysis. **Results:** Of 66 patients with OS, 19 (29%) of them presented with metastatic OS. The median age of diagnosis was 12.1 years with 5-year overall survival of 61.7% (95% CI, 48.1 to 75.3). The 5-year overall survival for those with non-metastatic and metastatic OS was 73.1% (95% CI, 58.1 to 88.1) and 34.7% (95% CI, 8.7 to 60.7, $P = 0.007$) respectively. The 5-year overall survival for those treated as per the MSKCC T12 and EOI was 72.4% (95% CI, 52.6 to 92.2) and 54.3% (95% CI, 36.3 to 72.3, $P = 0.087$) respectively. After controlling for confounding factors, patients with non-metastatic OS had higher 5-year EFS (HR, 0.228, 95% CI, 0.096 to 0.541, $P = 0.001$) and overall survival (HR, 0.294, 95% CI, 0.121 to 0.713, $P = 0.007$) compared to those with metastatic OS. Non-metastatic OS patients treated as per EOI regimen had lower 5-year EFS (HR, 2.397, 95% CI, 1.012 to 6.678, $P = 0.047$) compared to those treated per MSKCC T12 regimen. **Conclusion:** Multidrug combination chemotherapy including high-dose methotrexate (HD-MTX) and a multidisciplinary team approach introduced in 2003 in Singapore is well tolerated and can be safely delivered. The survival benefit between the 2 regimens still needs to be explored.

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Key words: Primary bone tumour, Singapore

¹Division of Paediatric Haematology-Oncology, KK Women's and Children's Hospital Singapore

²SingHealth Centre for Health Services Research, Singapore

³Division of Paediatric Haematology/Oncology, National University Hospital, Singapore

⁴Department of Orthopaedic Surgery, Hand and Reconstructive Microsurgery, National University of Singapore, Singapore

Address for Correspondence: Dr LeLe Aung, Parkway Cancer Centre, 6A Napier Road, Gleneagles Hospital, Level 3, Singapore 258500.

Email: aungl@pcc.sg

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Introduction

Osteogenic sarcoma (OS) is a primary malignant tumour of the bone, derived from primitive bone-forming mesenchyme and characterised by the production of osteoid tissue or immature bone by the malignant proliferating spindle cell stroma.¹⁻² Although primary bone tumours are rare in childhood, they are the sixth most common malignant neoplasms in children; in adolescents and young adults, they are the fourth most common malignant neoplasms, exceeded only by leukaemia, lymphomas and brain tumours.

There are 600 new cases per year reported in the United States but it occurs at a much lesser rate in Singapore. Its incidence rates among individuals under 24 years were 3.9 per million in men and women in Singapore.³ A Singapore Cancer Registry report by Shanmugaratnam K et al cites the incidence of OS over a 20-year period in Singapore as 20 cases per year.⁴

Since the introduction of multidrug regimen in the late 1970s, survival from localised OS elsewhere in the world was estimated at 60% to 78%.⁵⁻¹¹ More than 80% of patients

¹Division of Paediatric Haematology-Oncology, KK Women's and Children's Hospital Singapore

²SingHealth Centre for Health Services Research, Singapore

³Division of Paediatric Haematology/Oncology, National University Hospital, Singapore

⁴Department of Orthopaedic Surgery, Hand and Reconstructive Microsurgery, National University of Singapore, Singapore

Address for Correspondence: Dr LeLe Aung, Parkway Cancer Centre, 6A Napier Road, Gleneagles Hospital, Level 3, Singapore 258500.

Email: aungl@pcc.sg

diagnosed with a primary, localised OS develop recurrent disease primarily in the lungs within the first 2 years after diagnosis. These patients subsequently succumbed to the disease. For approximately 30% of those who presented with pulmonary metastatic disease at the time of initial diagnosis, survival remains dismal at about 20%. Prior reports revealed that more than 95% of those who died had lung metastasis as indicated by autopsy at the time of death.^{1,5} Although there is one report from Singapore on the survival experience with limb salvage emphasising the use of vascularised bone grafts and autoclaved tumour bone, we are not aware of any published studies on the various chemotherapy treatments given and the long-term outcomes for these young patients.¹² The current study was undertaken to report our experience on the clinical characteristics and survival outcome of these young patients with osteosarcoma treated in 2 major public hospitals, the KK Women and Children's Hospital (KKH) and the National University Hospital (NUH), Singapore pre- and post-multidrug regimen with high-dose methotrexate (HD-MTX).

Materials and Methods

Patients

A comprehensive list of patients who presented to the Department of Paediatrics, KKH and the Departments of Paediatrics, Hand and Reconstructive Microsurgery, and Orthopaedics, NUH with newly diagnosed, high-grade OS was generated. The detailed medical record of each patient was thoroughly reviewed. Inclusion was restricted to patients first seen in KKH and NUH between January 1994 and October 2011. Every attempt was made to include all patients seen during the study period. The former date was chosen arbitrarily as a date by which the earliest KKH and NUH census (1994 onwards) recorded the first patient diagnosed with OS. The latter date was chosen to allow adequate time for follow-up at the time of the analysis. There were 72 patients with primary OS seen and treated in KKH and NUH during the study period. Patients who were seen only for an initial consultation and were lost to follow-up or patients whose medical records were not available ($n = 5$) and 1 patient whose histology review resulted in liposarcoma, were excluded in this analysis. Analysis is based on the 66 remaining patients.

Treatment

All patients received chemotherapy and surgery for the treatment of their OS. During the time period from January 1994 to June 2003, the chemotherapy protocol used in NUH for the treatment of primary OS was according to the European Osteosarcoma Intergroup (EOI).^{6,13} The chemotherapy protocol used in KKH throughout the

study period until June 2006 for the treatment of primary OS was also according to the EOI regimen. The EOI protocol consisted of 2-drug combination chemotherapy and surgery totalling approximately 24 weeks with some variations in the actual treatment period. Chemotherapy agents included a combination of cisplatin (100 mg/m^2) given intravenously over 4 hours and doxorubicin (75 mg/m^2) with continuous infusion given intravenously over 72 hours every 3 to 4 weeks. Surgery, either limb salvage or amputation at the discretion of the treating surgeon, was followed by 2 to 3 cycles of adjuvant chemotherapy. In NUH, from April 2003 onwards, the chemotherapy protocol used was adapted from the Memorial Sloan-Kettering Cancer Centre's (MSKCC) T12 regimen and the Children's Oncology Group (COG) regimen.⁸⁻¹⁰ Multi-agent chemotherapy per cycle consisted of a combination of cisplatin (120 mg/m^2) given intravenously over 4 hours with doxorubicin (75 mg/m^2) given intravenously over 72 hours via continuous infusion; HD-MTX (12 g/m^2) was given intravenously over 4 hours with folinic acid (10 mg) given intravenously every 6 hours, first dose at hour 24 from the start of methotrexate (MTX); and ifosfamide (1.8 g/m^2) given intravenously over 1 hour for 5 days with etoposide (100 mg/m^2) given intravenously over 1 hour for 5 days. The treatment spanned over approximately 40 weeks with slight variation in the time required to complete the courses (Table 1). Surgery for all patients consisted of either limb salvage or amputation at the discretion of the treating surgeon and the team. Limb salvage surgical procedures at NUH were performed by using autoclaved tumour bone with vascularised bone graft or cadaver allograft bone as described previously, in addition to customised prostheses.¹² Patients with pulmonary metastatic disease underwent thoracotomies either immediately after definitive surgery of the primary tumour or at the end of their chemotherapy treatment. Treatment of subsequent relapses consisted of various chemotherapy regimens alone or surgery with or without additional individualised chemotherapy at both the centres. In some cases, high doses of radiation therapy were used.

Statistical Analysis

All analyses were performed using IBM SPSS 19.0. Descriptive statistics for quantitative variables were presented as median (range) and frequency (%) for qualitative variables. Adverse event-free survival (EFS) was defined as the endpoint in this study. This was defined as the time of entry into the study or time at diagnosis to the disease progression, occurrence of a metastatic disease, death from non-disease causes, development of a second malignant neoplasm, or last follow-up. The 5-year disease-free and overall survivals were calculated using

Table 1. Chemotherapy Schema

Chemotherapy	Days						
	1	2	3	4	5	21	28
EOI							
CD x 6 (course 1-6) every 3 weeks							
Cisplatin 100 mg/m ² /d by 4-hour infusion	x						
Doxorubicin 25 mg/m ² /d by 24-hour infusion	x	x	x				
Surgery after course 3							
T12 (non-metastatic)							
CD-M x 4 (courses 1, 2, 3, 5)							
Cisplatin 60 mg/m ² /d by 4-hour infusion	x	x					
Doxorubicin 25 mg/m ² /d by 24-hour infusion	x	x	x				
Methotrexate 12 g/m ² /d by 4-hour infusion						x	x
IE x 2 (courses 4, 6)							
Ifosfamide 1.8 g/m ² /d by 1-hour infusion	x	x	x	x	x		
Etoposide 100 mg/m ² /d by 1-hour infusion	x	x	x	x	x		
Surgery after course 2							

CD: Cisplatin, doxorubicin; CD-M: Cisplatin, doxorubicin, methotrexate; EOI: European Osteosarcoma Intergroup (cisplatin, doxorubicin); IE: Ifosfamide, etoposide; T12: High-dose methotrexate, cisplatin, doxorubicin, ifosfamide, etoposide

the method of Kaplan-Meier analysis.¹⁴ Cox proportional hazards regression model was performed to compare the disease-free and overall survivals adjusting for metastatic, non-metastatic disease and chemotherapy regimens. Statistical significance was set at $P < 0.05$.

Results

Patient Characteristics

Seventy-two patients were identified with a diagnosis of primary high-grade OS during the study period from January 1994 to October 2011. Six patients were excluded in this analysis as discussed above. Of the remaining 66 patients, the median age at diagnosis of primary OS was 12.1 years (range, 5.2 to 19.8 years), 26 patients were female (29%) and 40 were male (61%). The median follow-up for the entire cohort was 3.1 years (range, 0.3 to 13.3 years). The distributions of the initial site of OS were as follows: femur in 39 (59%) patients (31 distal), tibia in 15 (23%) patients, humerus in 9 patients (14%), 1 case each of clavicle and thoracic vertebrae, and the site of primary tumour was unknown in 1 patient. Only 1 patient presented with primary OS in the axial skeleton, in the thoracic vertebrae without any pulmonary involvement. Forty-seven (72%) patients presented with localised non-metastatic primary OS without any detectable pulmonary metastasis on imaging by chest x-ray (CXR) and/or computerised tomography (CT) scan. Of the 19 patients who presented with metastatic OS

at diagnosis, 16 were to the lungs (8 = bilateral), 1 to multiple bones (lumber L2-3 and ribs), 1 to scapula, and 1 patient presented with skip metastasis along the femur. The patient and tumour characteristics of all patients with OS are shown in Table 2.

Surgery

In patients who received chemotherapy as per the EOI protocol, 2 to 3 courses of neoadjuvant chemotherapy, every 3 to 4 weeks were delivered prior to undergoing surgical resection of the primary tumour. For those treated as per the MSKCC T12 regimen, surgery was performed at approximately 10 weeks from the start of chemotherapy. Sixteen (24%) patients underwent amputation as the surgical procedure for local control. Two patients did not have any surgeries for their primary tumours, whilst 1 patient's local control procedure mode was unknown. The latter patient died of disease at 1.4 years from diagnosis. A total of 47 (71%) patients, both from KKH and NUH underwent limb salvage procedures for their primary tumour. Of the 47 patients who underwent a limb salvage procedure, 15 of them who were from NUH underwent autoclaved tumour bone with vascularised bone graft as described previously. Of all the patients who underwent this limb salvage procedure at NUH, only 1 patient had a local recurrence. However, this patient presented with skip lesions at the time of initial diagnosis and underwent a limb salvage procedure against the treating surgeon's advice.

Table 2. Summary of Patient Characteristics

N = 66	No. of Subjects (%)
Age, Median (Range)	12.1 years (range, 5.2 to 19.8 years)
Follow-up, Median (Range)	3.1 years (range, 0.3 to 13.3 years)
Gender	
Male	40 (61%)
Female	26 (39%)
Site of primary OS	
Femur	39 (59%)
Tibia	15 (23%)
Humerus	9 (14%)
Other	3 (4%)
Metastatic	
Yes	19 (29%)
No	47 (71%)
Type of surgery	
Amputation	16 (24%)
Limb salvage	47 (71%)
Unknown/none	1/2 (5%)
Chemotherapy	
EOI	32 (48%)
T-12 like	33 (50%)
Unknown	1 (2%)
Relapse/progressive disease	
Yes	21 (32%)
Status	
ANED	36 (55%)
DOD	23 (35%)
AWD	5 (7%)
Unknown/(1 dead-other)	2 (3%)

ANED: Alive no evidence of disease; AWD: Alive with disease; DOD: Dead of disease; EOI: European Osteosarcoma Intergroup (cisplatin, doxorubicin); OS: Osteogenic sarcoma; T12: High-dose methotrexate, cisplatin, doxorubicin, ifosfamide, etoposide

Biologic Prognostic Factors

Of 66 patients, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) values at diagnosis were available for 31 (51%) of them. Our laboratory's reference normal ranges for LDH and ALP are 250-580 U/L and 40-130 U/L respectively. Six patients presented with LDH levels of more than 1000 U/L whilst 8 presented with levels more than the normal but less than 1000 U/L. Of the 14 patients who presented with higher than the upper limit of normal levels of LDH and ALP, 7 were alive and 5 were dead of the disease. Huvos histological necrosis grading was not available in 22 patients. Huvos histological grading was defined as grade 1 with necrosis $\leq 50\%$; grade 2 with necrosis $>50\%$ $<90\%$; grade 3 with necrosis $>90\%$ $\leq 99\%$; and grade 4 with necrosis of 100%.¹ Huvos histological necrosis grading following preoperative chemotherapy was

available for 44 of 66 patients who received neoadjuvant chemotherapy before definitive surgery for their primary OS (Table 3). Of the 44 patients whose tumour histological necrosis was available, 14 of 28 patients' tumours with grade 1 to 2 and 10 of 16 patients' tumours with grade 3 to 4 were alive with no evidence of disease.

Relapsed Patients

Salvage therapy for relapsed patients consisted of surgery with or without additional individualised chemotherapy. Of the 4 patients who were alive with no evidence of disease with relapsed OS to the lung, salvage therapy consisted of thoracotomy plus a combination of the following: ifosfamide (3 g/m²/day x 5 days) and muramyl tripeptide (MTP) (Patient 15); HD-MTX (12 g/m²/day) and ifosfamide (1.8 g/m²/

day x 5 days) (Patient 19); and MTX (8 g/m²); ifosfamide (1.8 g/m² x 5 days), etoposide (100 mg/m²/day x 5 days), cisplatin (120 mg/m²) and doxorubicin in 1 patient (Patient 21) (Table 3).

Outcome

The 5-year EFS and overall survival for the cohort were 57.7% (95% CI, 43.9 to 71.5) and 61.7% (95% CI, 48.1 to 75.3) respectively (Figs. 1, 2). The 5-year EFS for those with non-metastatic OS was 69.3% (95% CI, 54.5 to 84.1), while for those with metastasis at diagnosis was 14.1 (95% CI, 0.0 to 38.1), $P = 0.001$ (Fig. 3). The 5-year overall survival for those with non-metastatic OS was 73.1% (95% CI, 58.1 to 88.1) while for those with metastatic OS at diagnosis was 34.7% (95% CI, 8.7 to 60.7) $P = 0.007$ (Fig. 4). The 5-year EFS was 62.9% (95% CI, 41.9 to 83.9) per MSKCC T12 and 50.4% (95% CI, 31.2 to 69.6), $P = 0.171$ for patients who were treated as per the EOI regimen (Fig. 5). The 5-year overall survival was 72.4% (95% CI, 52.6 to 92.2) for patients who were treated as per the MSKCC T12 protocol versus 54.3% (95% CI, 36.3 to 72.3) for those treated as per EOI, $P = 0.087$ (Fig. 6). However, they

were not statistically significant even though MSKCC T12 protocol appeared to have longer time to occur to events. After controlling for potential confounding factors, when Cox proportional hazards regression model was applied, patients with non-metastatic OS had higher 5-year EFS (HR, 0.228, 95% CI, 0.096 to 0.541, $P = 0.001$) as compared to those with metastatic OS. Patients treated as per EOI regimen had lower 5-year EFS (i.e. shorter time to have event) (HR, 2.397, 95% CI, 1.012 to 6.678, $P = 0.047$) as compared to those treated as per the MSKCC T12 regimen (Fig. 7). In Cox regression model, patients with non-metastatic OS had higher 5-year overall survival (HR, 0.294, 95% CI, 0.121 to 0.713, $P = 0.007$) as compared to those with metastatic OS (Fig. 8). However, chemotherapy regimens for MSKCC T12 versus EOI for 5-year overall were no longer statistically significant in the model (HR, 2.588, 95% CI, 0.987 to 6.782, $P = 0.053$).

At the time of the analysis, median follow-up for the entire cohort from the time of diagnosis was 3.1 years (range, 0.3 to 13.3 years). Twenty-three (35%) were dead of disease. Forty-one (62%) patients were alive of which 36 (55%) were alive with no evidence of disease and 5 (7%)

Table 3. Patient and Tumour Characteristics of 21 Patients with Progressive or Relapsed Disease

No.	Age/Sex DX	Primary Site	Metastatic Site	Relapse Site	Therapy 1°/ Relapse	Type of Surgery	Huvos Grade	Dx-FU (Years)	Dx-R1 (Years)	Status
1.	14.8/F	Femur	Lung	Lungs	EOI	AKA	II	0.6	0.5	DOD
2.	13.3/M	Femur, D	None	Lungs, B	EOI	AKA	I	0.8	0.6	DOD
3.	11.6/F	Humerus, P	None	Lungs, B	EOI	AKA	NOS	0.9	0.5	DOD
4.	9.0/F	Tibia, D	None	Lungs, B	EOI	Salvage	III	1.6	1.1	DOD
5.	6.9/F	Femur, D	None	Lungs	EOI	AMP		1.7	1.5	DOD
6.	12.8/M	Tibia, P	Lungs	Lungs	EOI	AKA	NOS	1.8	0.3	DOD
7.	12.8/M	Femur, D	Skip lesions	Thigh, L	EOI	Salvage	I	1.8	1.8	DOD
8.	13.7/M	Femur	None	Lungs, Humerus	EOI	Salvage	NOS	2.1	1.1	DOD
9.	15.2/M	Humerus	No	Lungs	T12	Salvage	III	2.3	0.9	DOD
10.	16.7/M	Femur, P	Lungs	Lungs	T12	Salvage	III	2.6	0.6	DOD
11.	10.9/M	Femur, D	Lungs	Lungs, B	T12	Salvage	II	2.7	0.4	AWD
12.	14.9/F	Tibia	None	Lung	EOI	Salvage	NOS	3.1	0.4	AWD
13.	19.8/M	Femur, D	None	Lungs	T12	Salvage	I	3.1	1.0	DOD
14.	8.7/F	Femur	Lungs, B	Lungs	T12	Salvage		3.7	1.9	DOD
15.	7.4/F	Femur	None	Lungs	T12	Salvage	II	3.9	1.8	ANED
16.	12.4/F	Femur, Shaft	Lungs, B	Lungs, B	EOI	Salvage	I	4.0	1.3	DOD
17.	6.9/F	Femur, D	None	Local	EOI	Salvage	I	4.8	1.9	DOD
18.	6.4/F	Femur, D	Lungs, B	Lungs	T12	Salvage	II	5.7	3.5	DOD
19.	13.4/F	Tibia	None	Lungs	T12	AKA	II	8.9	0.8	ANED
20.	6.9/M	Humerus, P	None	Local	EOI	AKA	I	12.5	0.6	ANED
21.	11.6/F	Tibia	Lungs, B	Lungs	EOI	Salvage	III	13.3	0.7	ANED

ANED: Alive no evidence of disease; AKA: Above knee amputation; AMP: Amputation; AWD: Alive with disease; B: Bilateral; D: Distal; DOD: Dead of disease; Dx: Diagnosis; Dx-FU: Time diagnosis to follow up; Dx-R1: Time diagnosis to relapse; EOI: European Osteosarcoma Intergroup (cisplatin, doxorubicin); F: Female; M: Male; NOS: Not otherwise specified; P: Proximal; T12: High-dose methotrexate, cisplatin, doxorubicin, ifosfamide, etoposide; 1°: Primary tumour

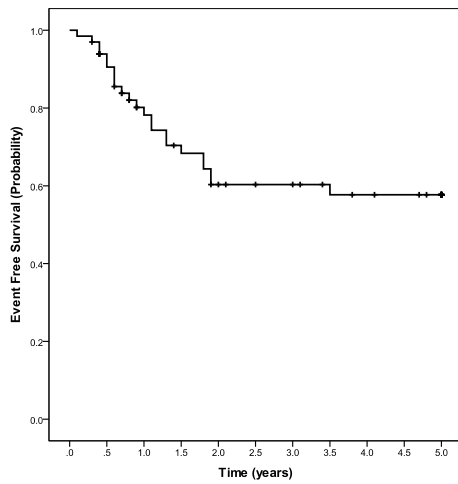


Fig. 1. Graph showing the 5-year event-free survival in patients with osteogenic sarcoma (n = 66).

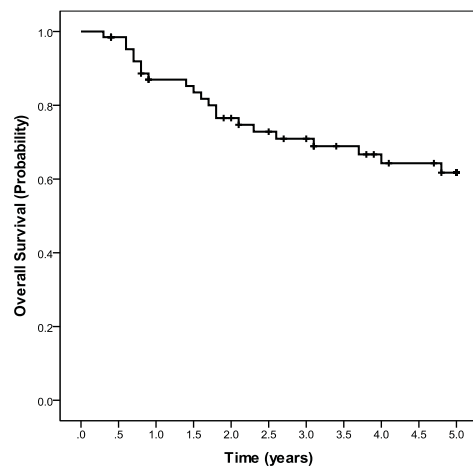


Fig. 2. Graph showing the 5-year overall survival in patients with osteogenic sarcoma (n = 66).

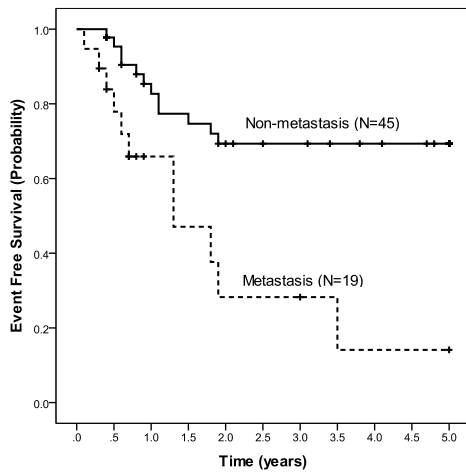


Fig. 3. Graph showing comparison of the 5-year event-free survival between non-metastatic versus metastatic osteogenic sarcoma in children patients.

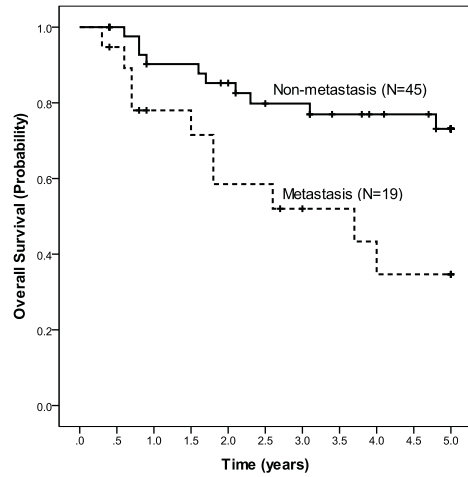


Fig. 4. Graph showing comparison of the 5-year overall survival between non-metastatic versus metastatic osteogenic sarcoma in children patients.

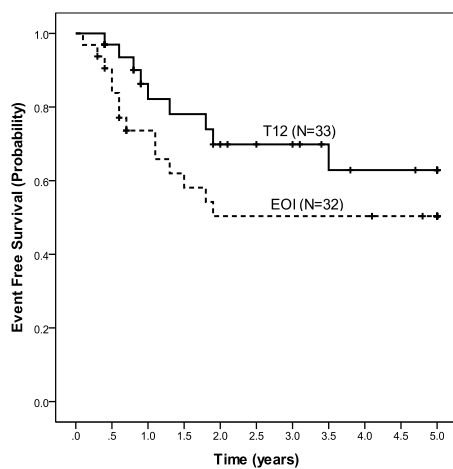


Fig. 5. Graph showing comparison of the 5-year event-free survival by chemotherapy EO1 versus T12 in children with osteogenic sarcoma.

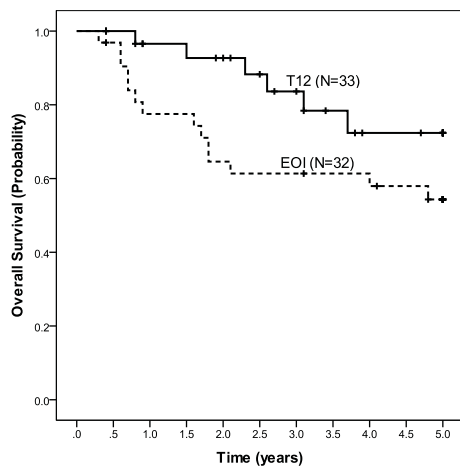


Fig. 6. Graph showing comparison of the 5-year overall survival by chemotherapy EO1 versus T12 in children with osteogenic sarcoma.

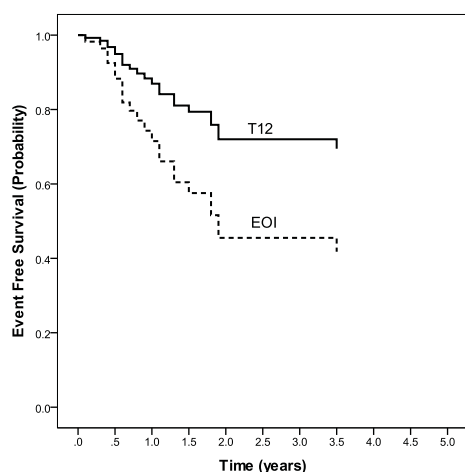


Fig. 7. Graph showing comparison of the 5-year event-free survival by chemotherapy EOI versus T12 in children with osteogenic sarcoma with the adjustment of metastasis status (Cox proportional hazards model).

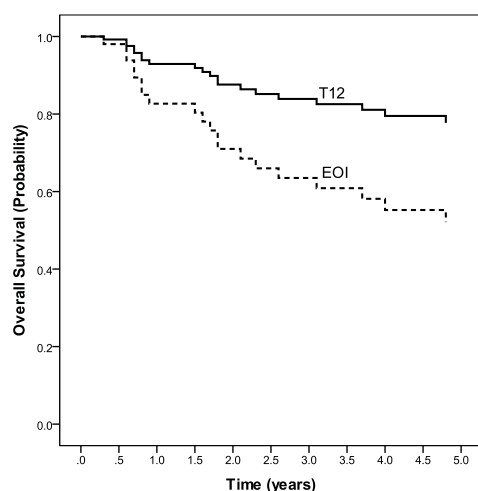


Fig. 8. Graph showing comparison of the 5-year overall survival by chemotherapy EOI versus T12 in children with osteogenic sarcoma with the adjustment of metastasis status (Cox proportional hazards model).

were alive with disease. One patient died of secondary leukaemia. This patient had thyroid carcinoma followed by OS and later succumbed to her third malignancy, leukaemia which occurred 4 years after completion of treatment of her OS. Of the 19 patients who presented with metastatic disease at diagnosis, 11 were dead of disease, 4 were alive with no evidence of disease, and 3 were alive with disease at the time of analysis. Twenty-one (32%) of 66 patients relapsed or had progressive disease at a median of 0.9 years (range, 0.3 to 2.5 years). All except 3 were relapses to the lung. Three patients had local recurrence after limb salvage procedure and 1 relapsed locally after amputation of the primary tumour in the humerus (Table 3). This latter patient's tumour achieved a grade 1 histological necrosis (Patient 20). One patient relapsed locally in the femur at 1.8 years from the initial diagnosis (Patient 7). This patient

presented with skip metastasis at diagnosis and underwent a limb salvage procedure at the parents' request against the surgeon's advice, with positive margins at the time of definitive surgery. Four of the 21 relapsed patients were alive with no evidence of disease at the time of analysis from 3.9 to 13.3 years from diagnosis with interval from diagnosis of primary to time of development of relapsed OS between 0.7 to 1.8 years.

Discussion

Survival from primary localised OS elsewhere in the world is estimated at 60% to 78% at 5 years.^{6-11,13} We describe here the patient and tumour characteristics in children and young adults diagnosed with OS and demonstrate an improvement in survival when a multidrug regimen incorporating HD-MTX was instituted in 2003.

OS fortunately is a relatively rare cancer in our Southeast Asian children and young adults.^{3,4,12} The most common site of presentation of the primary tumour in our series was the distal femur at 59% similar to that reported elsewhere in the world. There was a slight male predominance occurrence with a median age of presentation at 12.1 years. The younger age of presentation in our series may likely be a consequence of our analysis which was restricted to the 2 major paediatric hospitals where the upper age limit for paediatric patients is 15 years. However, there are similar reports of younger age of presentation in Asian/Pacific Islanders.⁴ Seventy-seven percent of the patients were of Chinese ethnicity, which represented the majority of the population in Singapore. The remainder of the population consists of the Malays and the Indians at a lesser percentage which was also similar to the ethnic distribution of the disease. We also found in our series, similar to elsewhere in the world, that approximately one-third of the patients presented with evidence of pulmonary metastatic disease on computed tomography (CT) scan at the time of initial diagnosis.

Serum prognostic factors ALP and LDH levels were only routinely obtained at the time of diagnosis in 31 patients. Of the 14 with high levels of these biological enzymes markers, only 5 were alive. Although our numbers are too small to make a concrete conclusion, as previously reported in larger series, there was an inverse relationship between survival and the level of ALP and LDH.^{7,8} We now routinely obtain ALP and LDH at the time of diagnosis and follow-up serially. Huvos histological necrosis was also not consistently and routinely reported in all our patients. Although we were limited by the incompleteness of histological grading information for all our patients' tumours, a higher tumour necrosis grade correlated with a better survival. However, in resource limited countries, efforts may be better allocated towards combating treatment abandonment and supportive care.

There were a total of 3 local relapses in our cohort, 2 of whom underwent limb salvage procedures for their primary tumour extirpation. The limb salvage technique in NUH has long been based on the biological technique, with the use of vascularised bone grafts in combination with autoclaved tumour bone, details of which were described previously by Pho et al.¹² In our analysis, the use of vascularised bone grafts in combination with autoclaved tumour bone did not appear to contribute to local recurrence in this small cohort of patients. This technique has also resolved the issue of limited resources in the region and the financial hardships which families face with high cost of customised prosthesis which are not covered by insurance or subsidies. Present analysis also revealed 21 patients who relapsed at a median of 0.9 years (range, 0.3 to 3.5 years) from the diagnosis of the primary tumour. There was little difference between the relapse rates and the site of relapse (lungs, in the majority of the patients) when patients who underwent amputation and limb salvage procedures were compared. However, the numbers were too small to detect whether there is a true significant difference.

In Singapore, for many years, the EFS and overall survival for patients with localised OS have been extremely poor at 40%. Elsewhere in the world, since the 1990s, survival rates for localised OS have plateaued in the range of 70% since the introduction of multidrug regimens incorporating HD-MTX. Modern management for children and young adults with OS has evolved mainly due to: multi-agent chemotherapy; better diagnostic imaging with multiplanar CT, magnetic resonance imaging (MRI) and positron emission tomography (PET) scans; aggressive surgery with advanced surgical techniques; and excellent supportive care (antibiotics, nutrition, anaesthesia, biomedical engineering, and rehabilitation). Radiation therapy can be considered if surgery is not an option in selected cases such as vertebral column, brain, and pelvis. However, doses of more than 50-70 Gy must be given in conjunction with aggressive chemotherapy.¹⁵ Many centres also use irradiation as an additional palliative treatment. In Singapore multi-agent therapy based on MSKCC T12 regimen with HD-MTX was introduced only in the mid-2000s. The dose of cisplatin given in each course was increased to 120 mg/m²/cycle (previous dosing at 100 mg/m²/cycle) given over 4-hour infusion on 2 separate days. Although Ferrari et al in their randomised trial reported that the addition of ifosfamide onto the cisplatin/doxorubicin/MTX backbone did not improve survival outcome, our regimen incorporated ifosfamide and etoposide into the adjuvant phase.¹⁶ The promising role of ifosfamide combined with etoposide in OS was demonstrated initially by Miser et al and Gentet et al in separate studies in relapsed OS patients.^{17,18} This combination was later studied in a larger trial conducted

by the Paediatric Oncology Group (POG) revealing 28 responses out of 39 newly diagnosed metastatic OS patients.¹⁹

This multidrug regimen together with advances in technology and excellent supportive care have allowed us to achieve improved survival rates of up to 70% reaching close to the world standards, for our young patients. It is important to note that there was no treatment-related toxic deaths throughout both the EOI and the T12 regimen eras. All patients succumbed to the disease. Hence, it is unlikely that this marginal higher survival was contributed in part by improved supportive care during the 2 treatments. The improved supportive care in the year 2000 onwards did indeed permitted safe delivery of multidrug intensive chemotherapy regimens such as T12. Albeit more intensive chemotherapy and a higher haematologic toxicity, this improved survival was clinically significant.

The field of paediatric oncology is evolving rapidly. It is in our best interest for the children to keep up-to-date with the current scientific discoveries, clinical trials results, and to practise best evidence-based medicine. Cure for children diagnosed with OS is possible when basic principles of therapy are applied. The key is to develop a committed and dedicated multidisciplinary team consisting of musculoskeletal oncology orthopedic surgeons, paediatric oncological surgeons, allied health members, and parents with clear goals. Secondly, we must all understand and agree that complete surgical extirpation of primary tumor is essential in both non-metastatic and metastatic disease. Despite two-thirds of patients succumbing to the disease, limb salvage rather than amputation, should be explored whenever feasible. Thirdly, effective systemic therapy must be delivered on strict schedule and without dose modification. It will be in our best interest to continue to visit whether a more intensive, stronger chemotherapy drug combinations and longer duration is in fact better than a simple two-drug regimen in a multicentre randomised controlled clinical trial settings. Lastly, the EFS and overall survival is not altered if macroscopic surgical resection is not done for metastatic and all clinically detectable disease, especially in those with pulmonary lesions. In conclusion, identifying and formulating optimal treatment strategies is complex and challenging for a child with both non-metastatic and metastatic OS. New drugs such as bisphosphonates, interferon, interleukin, and monoclonal antibodies have shown some encouraging results.²⁰⁻²² Biology studies, especially in our unique setting may identify prognostic factors.

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Neonatal Diabetes in a Singapore Children's Hospital: Molecular Diagnoses of Four Cases

Rashida F Vasanwala, ¹MBBS, MD (Paeds), MRCPCH (UK), Song Hai Lim, ²MBBS (Malaya), MRCPCH (UK), Sian Ellard, ³PhD, FRCPath, Fabian Yap, ¹MMed (Paeds), FRCPC (UK), FAMS

Abstract

Introduction: Neonatal diabetes (ND) presents below 6 months of age, and is caused by a genetic defect in glucose homeostasis. Molecular genetic diagnosis can identify the exact molecular aetiology and guide clinical management. The objective of this study was to identify ND among children with diabetes in a major children's hospital in Singapore and to characterise their molecular and clinical features. **Materials and Methods:** The study identified all infants below 6 months of age who presented with diabetes to our centre from January 2008 to December 2010. It also reviewed diabetes database comprising 662 patients, to identify those who were diagnosed with diabetes below 6 months of age between January 1997 and December 2010. Four patients (3 females and 1 male) were identified and their molecular aetiology was investigated. **Results:** A molecular aetiology was found in each of the 4 patients identified. Two patients (Patient 1 and 2) had permanent ND (PND). Patient 1 who has *KCNJ11*/R201H mutation was successfully switched from insulin to oral glibenclamide and Patient 2 who has a novel mutation *INS*/C109Y continues to be treated with insulin. Two patients (Patient 3 and 4) had transient ND (TND) and no longer require insulin or any other intervention to maintain normoglycaemia. Patient 3 has a novel mutation *ABCC8*/F1182S and Patient 4 has a paternal duplication on chromosome 6q24. **Conclusion:** This study identified 4 cases of ND in our cohort of diabetes children and confirmed their molecular diagnosis. Molecular genetic testing for these children led to accurate diagnosis and appropriate management.

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Key words: Monogenic diabetes, Permanent, Transient

¹Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore

²Department of Paediatrics, Putrajaya Hospital, Malaysia

³Department of Molecular Genetics, Royal Devon & Exeter NHS Foundation Trust, United Kingdom

Address for Correspondence: Dr Rashida F Vasanwala, Endocrinology Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899.

Email: rashidafv@hotmail.com

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Rashida F Vasanwala, ¹MBBS, MD (Paeds), MRCPCH (UK), Song Hai Lim, ²MBBS (Malaya), MRCPCH (UK), Sian Ellard, ³PhD, FRCPath, Fabian Yap, ¹MMed (Paeds), FRCPC (UK), FAMS

Abstract

Introduction: Neonatal diabetes (ND) presents below 6 months of age, and is caused by a genetic defect in glucose homeostasis. Molecular genetic diagnosis can identify the exact molecular aetiology and guide clinical management. The objective of this study was to identify ND among children with diabetes in a major children's hospital in Singapore and to characterise their molecular and clinical features. **Materials and Methods:** The study identified all infants below 6 months of age who presented with diabetes to our centre from January 2008 to December 2010. It also reviewed diabetes database comprising 662 patients, to identify those who were diagnosed with diabetes below 6 months of age between January 1997 and December 2010. Four patients (3 females and 1 male) were identified and their molecular aetiology was investigated. **Results:** A molecular aetiology was found in each of the 4 patients identified. Two patients (Patient 1 and 2) had permanent ND (PND). Patient 1 who has *KCNJ11*/R201H mutation was successfully switched from insulin to oral glibenclamide and Patient 2 who has a novel mutation *INS*/C109Y continues to be treated with insulin. Two patients (Patient 3 and 4) had transient ND (TND) and no longer require insulin or any other intervention to maintain normoglycaemia. Patient 3 has a novel mutation *ABCC8*/F1182S and Patient 4 has a paternal duplication on chromosome 6q24. **Conclusion:** This study identified 4 cases of ND in our cohort of diabetes children and confirmed their molecular diagnosis. Molecular genetic testing for these children led to accurate diagnosis and appropriate management.

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Key words: Monogenic diabetes, Permanent, Transient

Introduction

Neonatal diabetes (ND) is a rare form of diabetes affecting 1 in 100,000 to 300,000 live births. Its exact incidence is unclear, and it was only in recent years that ND has been recognised as an entity distinct in aetiology from type 1 diabetes mellitus (T1DM). Unlike patients with T1DM, patients who develop diabetes before 6 months of age do not have serological evidence of auto-immunity but instead have been found to carry mutations in one of a number of possible genes implicated in ND.¹⁻¹³ Known molecular aetiologies of ND now include mutations in the genes *KCNJ11* and *ABCC8* encoding the 2 protein subunits Kir6.2 and SUR1 of the ATP-sensitive potassium (K_{ATP}) channels respectively in insulin secreting beta-cells and mutations in

the insulin gene (*INS*), encoding insulin itself. Depending on the molecular aetiology, some children can be weaned off insulin therapy and achieve normal glucose control with oral sulphonylureas.¹⁴⁻¹⁶

ND may be transient (TND), resolving within a median of 3 months, or permanent (PND), in which case treatment is required for life. Each subgroup accounts for about 50% of the patients. Approximately 70% of TND is caused by the overexpression of certain genes in a region of the long (q) arm of chromosome 6 called 6q24,¹⁷ while about half of infants with PND have mutations in the *KCNJ11* gene. Infants with TND typically present in the first 4 weeks of life while those with PND develop diabetes later from 2 to 6 months of age.

¹Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore

²Department of Paediatrics, Putrajaya Hospital, Malaysia

³Department of Molecular Genetics, Royal Devon & Exeter NHS Foundation Trust, United Kingdom

Address for Correspondence: Dr Rashida F Vasanwala, Endocrinology Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899.

Email: rashidafv@hotmail.com

This knowledge of ND has important implications in patient management. By establishing the molecular aetiology of an infant with ND, it may be possible to predict the likelihood of insulin-dependency or of substituting insulin therapy with oral sulphonylureas. The molecular diagnosis is also important in genetic counseling. We conducted this study to identify children with ND among new and existing cases in our institution.

Materials and Methods

Patients

This study was conducted at KK Women's and Children's Hospital (KKH), an 830-bed tertiary hospital in Singapore. Only Singapore-born children were included in the study. Case-finding was conducted in the following ways:

- All children presenting with very early onset diabetes (6 months old and younger) who required insulin therapy from 1 January 2008 to 31 December 2010 were identified. Each child had the following investigations performed as part of the workup for newly diagnosed diabetes — blood gases, blood/urine for ketones, C-peptide and serum insulin levels, glutamic acid decarboxylase (GAD) and islet cell antibody (ICA) levels. Blood was sent for genetic testing.
- The hospital maintains a diabetes database which records all cases of diabetes between 0 to 16 years, with information on gender, date of birth, date of diagnosis, type of presentation and diabetes type. The database comprised 662 patients who sought treatment in our hospital between 1 January 1997 and 31 December 2010 at the time of this study. A review of the database was performed to identify those who were diagnosed with diabetes below 6 months of age. Their clinical data was extracted and parent's consent was obtained for genetic testing.

Mutational Analysis

Molecular genetic analysis was performed at the molecular genetics laboratory in Exeter, United Kingdom. Methylation-specific polymerase chain reaction (MSP) testing for chromosome 6q24-related TND was performed at the Wessex Regional Genetics Laboratory in Salisbury, United Kingdom. The methods used have been previously reported.¹³

Ethical Committee Approval

The study was approved by the hospital's Institutional Review Board. Informed consent was obtained from patients/parents for molecular testing.

Results

We identified 4 cases out of a total cohort of 662 patients over 13 years (1 January 1997 to 31 December 2010). Three patients were diagnosed over 2 years (1 January 2008 to 31 December 2009) and one was diagnosed retrospectively from the database.

Their clinical characteristics are presented in Table 1 and family pedigrees charts are shown in Figure 1.

- Patient 1 (diagnosed in June 2008), Patient 3 (diagnosed in November 2008) and Patient 4 (diagnosed in July 2009), were identified at time of diagnosis, while Patient 2 (diagnosed in 1994), was identified from the diabetes database review.
- Two of the children had PND — Patient 1 (onset 3 months old, converted to oral glibenclamide at age 6 months) and Patient 2 (onset 6 months old, still on insulin therapy 18 years on).
- The other 2 children had TND — Patient 3 (onset 3 weeks old, weaned off insulin at age 6 months) and Patient 4 (onset 3 days old, weaned off insulin at age 2 months). The 2 cases of TND presented relatively earlier within one month of life, required initial insulin treatment and later were successfully weaned off insulin.
- Two children had novel mutations — Patient 2 (C109Y in exon 3 of the *INS* gene) and Patient 3 (F1182S in exon 28 of the *ABCC8* gene). The other two children had common well-described genetic diagnoses — Patient 1 (R201H of the *KCNJ11* gene) and Patient 4 (paternal duplication of chromosome 6q24).

Patient 1

Patient 1 was 3 months old when she presented with infantile pyrexia and vomiting. Her full septic workup was negative. In view of hyperglycaemia, metabolic acidosis and ketonuria, a diagnosis of diabetic ketoacidosis (DKA) was made and intravenous insulin was started. She responded well to the treatment and her insulin was converted to subcutaneous insulatard and novorapid. In view of very early onset of presentation, a probable diagnosis of monogenic diabetes was considered and molecular genetic analysis confirmed a heterozygous missense mutation R201H in the *KCNJ11* gene. She has been successfully weaned off insulin and has excellent blood glucose control on glibenclamide twice daily.

Patient 2

Patient 2 was identified from the diabetes database. She was initially diagnosed with T1DM at 6 months of age in

Table 1. Clinical Characteristics of Singaporean Children Identified with Neonatal Diabetes

Clinical Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Sex	Female	Female	Male	Female
Race	Chinese	Chinese	Chinese	Chinese
At birth				
Weight (grams)	2145	NA	2800	2196
Gestation (weeks)	38	NA	38	37
At presentation				
Age	3 months	6 months	3 weeks	3 days
Presentation	Ketoacidosis	Ketoacidosis	Hyperglycaemia	Hyperglycaemia
Glucose (mmol/L)	20.0	NA	25.2	17.4
HbA1c (%)	14.5	NA	5	Not tested
Autoantibody	Negative	NA	Negative	Negative
C-peptide (ng/mL)	0.7	NA	0.5	0.4
Treatment initiated	Insulin injections	Insulin injections	Insulin injections	Insulin injections
Mutation	<i>KCNJ11/R201H</i>	<i>INS/C109Y</i>	<i>ABCC8/F1182S</i>	<i>Pat. Dupl. Chrom. 6q24</i>
Current status				
Age	4.5 years	18 years	3.5 years	3 years
Weight (kg)	17.4	61.1	7.7 (at 9 months)	18.6
Type of treatment	Glibenclamide	Insulin injections	Weaned off insulin	Weaned off insulin
HbA1C (%)	6.5	8.2	5.9	5.1
C-peptide (ng/mL)	2.2	0.3	0.6	Not tested
Type of ND	Permanent	Permanent	Transient	Transient

HbA1c: Glycated haemoglobin; NA: Not available; ND: Neonatal diabetes; *Pat. Dupl. Chrom. 6q24*: Paternal duplication of chromosome 6q24

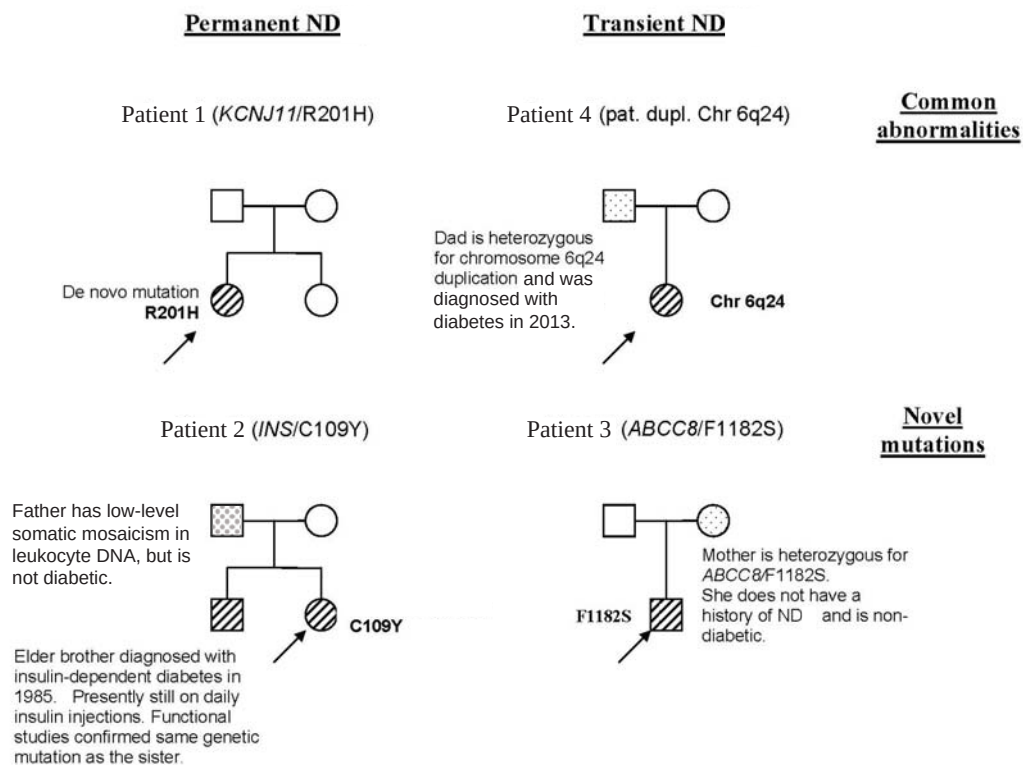


Fig. 1. Pedigree charts of Singaporean children identified with neonatal diabetes.

1994. She was found to have a novel mutation C109Y in exon 3 of the *INS* gene, which is consistent with a phenotype of PND. She has a 25-year-old brother also with insulin-dependent diabetes from infancy. Following the diagnosis of ND in Patient 2, genetic studies have been done on her brother confirming the same gene mutation. Father has low-level somatic mosaicism in leukocyte DNA, but is not diabetic.¹⁸

Patient 3

Patient 3 presented at 3 weeks of age with prolonged neonatal jaundice, and on further evaluation was found to have glycosuria and hyperglycaemia with no ketoacidosis. Molecular genetic analysis confirmed a heterozygous novel missense mutation, F1182S, in exon 28 of the *ABCC8* gene, which is consistent with a diagnosis of TND. The heterozygous mutation was inherited from his mother, who was non-diabetic and did not have a history of neonatal hyperglycaemia.

Patient 4

Patient 4 presented at 3 days of life with hyperglycaemia with no ketoacidosis. She was a term small for gestational age (SGA) baby with normal clinical examination except for macroglossia. Molecular genetic analysis confirmed a diagnosis of TND caused by paternal duplication of chromosome 6q24. Hypermethylation at this locus was present in patient's father, indicating that the father carries this duplication on his maternal chromosome.

Discussion

We report 4 cases of ND with variable clinical and molecular characteristics, identified by systematically examining new and existing patients with very early infancy-onset diabetes in our institution. Secondly, our study highlights the clinical characteristics of 2 children with novel missense mutations involving the *ABCC8*/F1182S and *INS*/C109Y genes, which expands the catalogue of known mutations that may cause TND and PND. Thirdly, our results illustrate the importance of accurate diagnosis as it has implications for treatment and prognosis.

The first case of PND (Patient 1) with missense mutation R201H in the *KCNJ11* gene represents the most common PND causing mutation. These mutations are familial, or more commonly, sporadic in nature.¹⁹ This heterozygous gain of function mutation in the *KCNJ11* gene encoding the Kir6.2 subunit results in ATP-insensitive channels that will respond to sulphonylurea with channel closure and insulin release. Clinical application of these drugs in ND with these mutations affecting the ATP sensitivity of the channel is reflected in this case. A successful introduction

of glibenclamide saw a rapid weaning in her insulin dose besides producing good glycaemic control. Recently, the efficacy of therapeutic amendment from insulin to sulphonylurea-based treatment was assessed in cases of neonates with diabetes owing to Kir6.2 mutations.¹⁵ Ninety percent of subjects had a successful therapeutic response to an oral sulphonylurea.¹⁵ Sulphonylureas were not only effective in achieving an acceptable level of glycated hemoglobin, but they also sustained the euglycemic response in patients with Kir6.2 mutations.¹⁵ Independently, other studies have also established similar success of sulphonylureas in patients with ND owing to Kir6.2 mutations.^{19,20} Oral sulphonylurea treatment thus forms an attractive alternative to lifelong exogenous injections of insulin in these patients.

The second case of PND (Patient 2) has novel mutation C109Y in exon 3 of the *INS* gene. Patient 2 was diagnosed with T1DM 18 years ago when she was 6 months old — we have reclassified her diagnosis as PND following genetic confirmation. *INS* mutations have recently been described as the second commonest cause of PND and should be assessed in neonatal and early childhood “type 1-like insulin requiring diabetes”, particularly when autoimmune markers are absent.^{7,12,13} Most mutations are located within the A or B insulin chains which disrupt the folding of the pro-insulin molecule, resulting in a misfolded protein or retention of the protein in the endoplasmic reticulum (ER), causing ER stress and beta-cell apoptosis. Disulfide bonds are crucial for pro-insulin folding in the ER and 60% of the mutations either abolish or disrupt disulfide bridge formation by substitution or addition of a cysteine residue.^{21–23} The majority of patients with *INS* gene mutations are sporadic cases that result from de novo mutations, while some cases show autosomal dominant inheritance, which was implicated in this case. The clinical characteristics of patients with *INS*, *KCNJ11*, or *ABCC8* gene mutations were compared in the large Exeter cohort. No difference in sex, birth weight, or gestational age was observed between the 3 groups, but patients with an *INS* mutation were diagnosed later (median 11 weeks) than those with K_{ATP} channel mutations. Coincidentally, this patient did present at later age among the 4 cases of ND. Also, no neuropsychological or neuromotor dysfunction were present in children with an *INS* mutation in contrast to the children with a K_{ATP} channel mutations. Our patient is now 18 years old, both she and her 25-year-old biological brother continue to remain on subcutaneous insulin therapy. In summary, these mutations are the second most common cause of PND, and *INS* gene screening should be recommended in all children diagnosed with diabetes in the first year of life.

Our first case of TND (Patient 3) has a novel mutation *ABCC8*/F1182S and has not been previously reported. The *ABCC8*/F1182S mutation may result in TND by affecting

the SUR1 subunit of the K_{ATP} channel.²⁴ Although the molecular mechanisms of *ABCC8* and *KCNJ11* mutations are distinct, the cellular mechanism reducing insulin release is common to both. We speculate that the *ABCC8*/F1182S mutation was pathogenic because the phenylalanine at position 1182 is conserved across species. A different mutation (F1182L) has been identified in another patient with transient neonatal diabetes and neither mutation has been detected in 500 patients with the opposite phenotype of hyperinsulinism.²² In addition, the adjacent residue, R1183, is frequently mutated in patients with TND. The F1182S mutation was inherited from patient's unaffected mother. This case was challenging as with a mutation possible to be TND, it was subsequently proven when the patient was successfully weaned off insulin with no other treatment. As with *KCNJ11*, activating mutations in *ABCC8* encoding the SUR1 regulatory subunit of the K_{ATP} channels cause both permanent and transient neonatal diabetes.⁵ Mutations of *KCNJ11* are typically associated with PND, whereas most mutations of *ABCC8* are associated with TND, perhaps reflecting a less severe form of diabetes.

Our second case of TND (Patient 4) has a common molecular cause — paternal duplication of chromosome 6q24. She presented at day 3 of life with hyperglycaemia and required insulin to achieve normoglycaemia. Titrating and delivering small doses of insulin with feeds was a challenging issue. She was monitored closely and the insulin was weaned off completely with good glycaemic control. Chromosome 6q24-related TND begins in the first 6 weeks of life and resolves by 18 months of age. Intermittent episodes of hyperglycaemia may occur in childhood, particularly during intercurrent illnesses. Recurrence in adolescence is more akin to type 2 DM. Women are also at risk of relapse during pregnancy and present with gestational diabetes. The cardinal features are intrauterine growth retardation, dehydration and hyperglycaemia and absence of ketoacidosis. Macroglossia and umbilical hernias are often present. These features were seen in this case which helped to clinch the diagnosis in addition to molecular testing. This form of TND is caused by overexpression of 2 genes, *PLAGL1*(ZAC) and *HYMAI*, found within an imprinted region on chromosome 6q24. Molecular diagnosis is based on identifying an additional paternal copy of *PLAGL1*(ZAC)/*HYMAI* or loss of maternal methylation within the differentially methylated region (DMR) of the promoter of *PLAGL1*(ZAC)/*HYMAI*. Three known mechanisms cause TND: paternal uniparental disomy of chromosome 6, duplication of 6q24 on the paternal allele, and a 6q24 methylation defect.²⁵ The risk to siblings and offspring of a proband of having TND or developing diabetes later in life depends upon genetic mechanism in the family. TND caused by paternal uniparental isodisomy of

chromosome 6 is typically a de novo, non-recurrent event. Duplication of 6q24 (paternal) can be de novo, inherited in an autosomal dominant manner, or inherited as a part of a complex chromosome rearrangement. It may recur in siblings and offspring of a proband if the duplication is inherited from the father. All reported instances of individuals with a methylation defect have been simplex cases (i.e. a single occurrence in the family). However, it is possible that the offspring of individual with TND caused by methylation defect may be at risk of developing TND or diabetes in later life if his/her affected parent is female. Prenatal diagnosis of TND is possible in those individuals at risk for a structural chromosome abnormality.

Conclusion

Our study is the first report in Singapore describing a series of 4 confirmed cases of neonatal diabetes representing distinct clinical and genetic variability spectrum. Two novel mutations expand the catalogue of mutations that may cause ND. Our study highlights the need for medical practitioners to consider molecular testing for all patients who present with diabetes below 6 months of age as this will facilitate accurate diagnosis and guide appropriate therapy.

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Surgeon-Performed Ultrasound-Guided Fine-Needle Aspiration Cytology (SP-US-FNAC) Shortens Time for Diagnosis of Thyroid Nodules

Wei Xiang Gu,¹ Chuen Seng Tan,² BSc, MSc, PhD, Thomas WT Ho,³ MBBS, FRCSEd, FAMS

Abstract

Introduction: Ultrasound-guided fine-needle aspiration cytology (US-FNAC) of thyroid nodules is an important diagnostic procedure. In most hospitals, patients are referred to radiologists for US-FNAC, but this often results in a long waiting time before results are available. Surgeon-performed US-FNAC (SP-US-FNAC) during the initial patient consultation attempts to reduce the waiting time but it is not known whether this is as accurate as radiologist-performed US-FNAC (RP-US-FNAC). The aim of this study is to compare the clinical efficiency between SP-US-FNAC and RP-US-FNAC. **Materials and Methods:** A retrospective study was performed on patients from the Department of General Surgery, Tan Tock Seng Hospital (TTSH) who underwent an US-FNAC from August 2011 to May 2012. All cases of SP-US-FNAC were performed by a single surgeon. This study compared the rates of positive diagnoses achieved by SP-US-FNAC and RP-US-FNAC as well as the time interval to reach a cytological diagnosis by each group. **Results:** A total of 40 cases of SP-US-FNAC and 72 cases of RP-US-FNAC were included in the study. SP-US-FNAC resulted in 28 (70%) positive diagnoses and 12 (30%) non-diagnoses while RP-US-FNAC resulted in 47 (65.3%) positive diagnoses and 25 (34.7%) non-diagnoses. These results were comparable ($P = 0.678$). The median time taken to reach a cytological diagnosis was 1 working day for SP-US-FNAC and 29.5 working days for RP-US-FNAC resulting in a shorter interval to reaching a cytological diagnosis for SP-US-FNAC ($P < 0.001$). **Conclusion:** In the workup of thyroid nodules, SP-US-FNAC is as accurate as RP-US-FNAC but significantly reduces the time taken to reach a cytological diagnosis. This leads to greater clinical efficiency in the management of patients with thyroid nodules, which in turn leads to other benefits such as decreased patient anxiety and increased patient satisfaction.

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Key words: Accuracy, Biopsy, Diagnostic, Thyroid workup, Waiting times

¹Yong Loo Lin School of Medicine, National University of Singapore, Singapore

²Saw Swee Hock School of Public Health, National University of Singapore, Singapore

³Section of Head and Neck Surgery, Department of General Surgery, Tan Tock Seng Hospital, Singapore

Address for Correspondence: Dr Thomas Ho, Department of General Surgery, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433.

Email: Thomas_ho@ttsh.com.sg

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Abstract

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Introduction

Fine-needle aspiration cytology (FNAC) is an efficient and reliable diagnostic test that is important in the evaluation of thyroid nodules.¹ This is to exclude the possibility of a cancer, even though the vast majority of thyroid nodules are actually benign.² FNAC is known to have high rates of sensitivity, specificity and diagnostic accuracy, and this has been further improved with the introduction of ultrasound guidance in FNAC. Ultrasound-guided FNAC (US-FNAC) has been proven to be superior to palpation-guided FNAC in many studies worldwide.³⁻⁷ This is due to increased sensitivity and specificity, as well as decreased rate of false negatives and sample inadequacy. This ultimately decreases

the need for repeat FNAC, which incurs time and monetary cost to patients.⁶

US-FNAC has traditionally been performed by trained radiologists. This requires a referral from the clinician to the radiologist, and the procedure is often performed at a different date and venue. This additional visit inevitably results in patient's inconvenience and delay in making a diagnosis. However there has been an increasing shift towards surgeons performing US-FNAC.⁷⁻⁹ Recent studies have shown benefits of surgeon-performed US-FNAC (SP-US-FNAC), which include patient convenience by reducing the number of physician visits and direct feedback of information for surgeons, allowing for faster decision-

¹Yong Loo Lin School of Medicine, National University of Singapore, Singapore

²Saw Swee Hock School of Public Health, National University of Singapore, Singapore

³Section of Head and Neck Surgery, Department of General Surgery, Tan Tock Seng Hospital, Singapore

Address for Correspondence: Dr Thomas Ho, Department of General Surgery, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433.

Email: Thomas_ho@ttsh.com.sg

making on whether surgery is needed.^{7,9-11} The shift towards surgeon-based ultrasonography also helps in streamlining patient care and improving patient satisfaction.¹² SP-US-FNAC is a relatively new practice in Singapore, and to our knowledge, it has not been consistently offered by surgeons. The senior author is the first clinician to consistently perform US-FNAC in his practice at Tan Tock Seng Hospital.

To further assess the benefits of SP-US-FNAC, we conducted a retrospective study to compare the diagnostic accuracy between radiologist-performed US-FNAC (RP-US-FNAC) and SP-US-FNAC, and more importantly, to compare the time taken to reach a cytological diagnosis between radiologist and SP-US-FNAC.

Materials and Methods

We performed a retrospective review of 112 patients who underwent US-FNAC between 1 August 2011 and 31 May 2012 at Tan Tock Seng Hospital (TTSH). SP-US-FNAC was performed in the outpatient clinic by a single consultant surgeon from the Head and Neck Services of General Surgery Department, and RP-US-FNAC was performed by several radiologists from the Radiology Department. Information retrieved included patient's age, gender, nodule size, consistency of nodule and location, appearance of nodule on ultrasonography, date of first visit to the surgeon, cytology results, date of cytology results, as well as histology results for patients who subsequently underwent surgery. The information was retrieved from patient's case notes and online databases. Institutional Review Board approval was obtained prior to data collection.

Imaging Technique

Using a hand-held probe, the thyroid gland was imaged to determine the size and presence of nodules. Nodules were then examined with regard to size (in centimetres), consistency (solid, cystic or mixed solid-cystic), margins (well-defined or poorly-defined), echogenicity (hypoechoic, hyperechoic, isoechoic or anechoic), and for presence of microcalcifications. Internal vascularity was assessed using biphasic Doppler mode.

US-FNAC Technique

SP-US-FNAC was performed by one surgeon, using 23-gauge needles with no negative pressure, unless there were cystic contents that required aspiration. Needle placement into the nodule was visualised real-time on the ultrasound monitor, and multiple passes into each nodule were made. The cells were then expelled using a syringe onto a glass slide which was immediately smeared and placed in fixation medium. The surgeon then assessed

sample adequacy by visualising for cellular material against a light source, without the help of on-site cytotechnicians or cytologists. In some cases, more than one nodule was sampled from a single patient, in which some took place during the same visit and others during separate visits. RP-US-FNAC was commonly performed using 25-gauge needles with no negative pressure, and with the support of on-site cytotechnicians. The number of passes into each nodule was dependent on the on-site cytological evaluation.

Cytology Reporting

The Department of Pathology at TTSH analysed and reported the slides. The results were reported based on the Bethesda system.¹³ "Insufficient" samples may be further reported by the pathologists as "low cellular yield", "bloody" or "cystic contents". We considered any result that is not reported as "insufficient" as a positive diagnosis.

Time Taken to Reach Cytological Diagnosis

The time taken to reach a cytological diagnosis was calculated as the number of working days from the patient's first visit to the surgeon's clinic to the day which the cytological diagnosis was made available on the hospital's online reporting system by the reporting pathologist. A working day is taken to be any day that is not a Saturday, Sunday or public holiday. Patients who underwent more than one FNAC during the same visit were considered as a single case.

Statistical Analysis

Univariate analysis was performed using Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables. A *P* value <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS 20.

Results

A total of 130 US-FNAC studies were performed on 112 patients over the same time period. Of these, 40 patients underwent SP-US-FNAC and 72 patients underwent RP-US-FNAC. We excluded repeated visits within our study period. There were more patients in radiology due to hospital-wide referrals from multiple departments. Nevertheless, the age, gender, nodule size distribution and proportion of nodules with cystic components between both groups were comparable (Table 1).

Positive Diagnosis Rate

The positive diagnosis rate between SP-US-FNAC and RP-US-FNAC were comparable. SP-US-FNAC resulted in 28 (70%) positive diagnoses and 12 (30%) non-diagnoses while RP-US-FNAC resulted in 47 (65.3%) positive diagnoses and 25 (34.7%) non-diagnoses. The difference in inadequacy rate between SP-US-FNAC and RP-US-FNAC was not statistically significant ($P = 0.68$) (Table 2). Likewise, the non-diagnosis rate for nodules with cystic components was also not statistically significant between SP-US-FNAC and RP-US-FNAC ($P = 0.434$) (Table 3).

Time Taken to Establish Diagnosis

The median time taken to establish a cytological diagnosis was 1 working day for SP-US-FNAC (interquartile range, 1, 1) and 25 working days for RP-US-FNAC (interquartile range, 16, 39.8). There was a significantly shorter time taken in reaching a diagnosis for SP-US-FNAC ($P < 0.001$) (Table 2). All nodules which underwent SP-US-FNAC reached a cytological diagnosis within 2 working days. The time taken to diagnosis was plotted on the Kaplan-Meier curves (Fig. 1).

Table 1. Characteristics of Patients and Nodules

	SP-US-FNAC (n = 40)	RP-US-FNAC (n = 72)	P value
Patient's age (years), median (interquartile range)	45.5 (33.5, 61.3)	54 (41.5, 62.3)	0.099
Patient's gender			
Female	30 (75%)	58 (80.6%)	0.631
Male	10 (25%)	14 (19.4%)	
Size of nodule (cm), median (interquartile range)	3 (2.2,3.8)	2.8 (1.6,3.6)	0.183
Nodules with cystic component	21 (52.5%)	41 (56.9%)	0.695

RP-US-FNAC: Radiologist-performed ultrasound-guided fine-needle aspiration cytology; SP-US-FNAC: Surgeon-performed ultrasound-guided fine-needle aspiration cytology; US-FNAC: Ultrasound-guided fine-needle aspiration cytology

Table 2. Comparison of Positive Diagnosis Rate between SP-US-FNAC and RP-US-FNAC

	SP-US-FNAC (n = 40)	RP-US-FNAC (n = 72)	P value
Positive diagnosis rate	28 (70%)	47 (65.3%)	0.678
Time taken to reach a cytological diagnosis (working days), median (interquartile range)	1 (1,1)	29.5 (16,39.8)	<0.001

RP-US-FNAC: Radiologist-performed ultrasound-guided fine-needle aspiration cytology; SP-US-FNAC: Surgeon-performed ultrasound-guided fine-needle aspiration cytology; US-FNAC: Ultrasound-guided fine-needle aspiration cytology

Table 3. Comparison of Non-Diagnosis Rate for Nodules with Cystic Components between SP-US-FNAC and RP-US-FNAC

	Cystic nodules which underwent SP-US-FNAC (n = 21)	Cystic nodules which underwent RP-US-FNAC (n = 41)	P value
Non-diagnosis rate	11 (52.4%)	17 (41.5%)	0.434

RP-US-FNAC: Radiologist-performed ultrasound-guided fine-needle aspiration cytology; SP-US-FNAC: Surgeon-performed ultrasound-guided fine-needle aspiration cytology

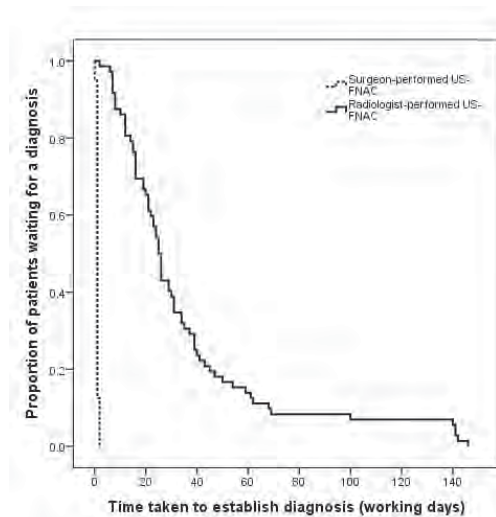


Fig. 1. Kaplan-Meier curves for the time taken to reach a cytological diagnosis as calculated from the patient's first visit at the surgeon's clinic to the time a cytological diagnosis is made by the Pathology Department based on the fine-needle aspiration cytology (FNAC) sample. Surgeon-performed ultrasound-guided fine-needle aspiration cytology (SP-US-FNAC) had a significantly shorter time to diagnosis ($P < 0.001$).

Discussion

In recent years, there has been much focus on evaluating the use of ultrasound in head and neck surgery, in particular the role of ultrasound-guidance in FNAC.^{4,7,14-16} US-FNAC, whether performed by radiologists or clinicians, has shown multiple benefits such as increased sensitivity and specificity, decreased inadequate specimen rate, increasing patient safety by visualising the needle tip and avoiding trachea and surrounding vessels, allowing for selection of the most suspicious-looking nodule or the solid part of cystic nodules to be sampled, and permitting FNAC of poorly felt nodules which may be small or posteriorly located.¹⁴ Bringing US-FNAC into the domain of clinicians further adds on to the above benefits. There is a reduction in the number of consultations, resulting in decreased time and monetary costs.^{11,12} Compared to radiologists, surgeons have the distinct advantage of being able to correlate ultrasound findings within the patient's clinical context.^{14,17} As such, US-FNAC performed by the surgeon is associated with a low sampling error rate.¹¹

The aim of our study was to determine: 1) whether the rate of positive diagnoses was comparable between SP-US-FNAC and RP-US-FNAC within a single institution, and if so, 2) whether there is a significant reduction in time taken to reach a cytological diagnosis for SP-US-FNAC. Our results show a comparable rate of positive diagnoses between SP-US-FNAC (70%) and RP-US-FNAC (65.3%), with a significantly reduced time taken to reach a diagnosis for SP-US-FNAC (1 working day compared to 25 working

days). Though the results of our study is limited by a small sample size, it indicates that a shift of US-FNAC from the domain of radiologists to surgeons can decrease the waiting time to diagnosis for patients without compromising on the rate of positive diagnosis.

Other authors have reported non-diagnostic rates of US-FNAC between 3.5% and 32%^{4,9} with an average rate of 10%.¹ It has been observed that institutions with higher case loads and more extensive experience have much lower non-diagnostic rates in the range of 5%.^{4,7,10} Our institution's higher non-diagnostic rates of 30% for SP-US-FNAC and 34.7% for RP-US-FNAC is likely due to a multitude of factors such as the inclusion of all patients who underwent US-FNAC within the study period and an initial lack of experience for SP-US-FNAC. The sampling of nodules with large cystic components and sending of cystic aspirates for cytological analysis using cell-block technique may also have contributed to the higher non-diagnostic rates.¹⁸

Many studies examining the role of immediate on-site cytological evaluation in thyroid FNAC have produced conflicting results.¹⁹⁻²³ Redman et al²⁰ and Baloch et al²¹ showed a significant improvement in diagnostic accuracy and decreased false-negative rates with the addition of on-site cytological evaluation to US-FNAC. On the other hand, a study by O'Malley et al which specifically investigated the differences between cytological evaluation in US-FNAC concluded that immediate on-site cytological evaluation only increased procedure time without a concomitant increase in specimen adequacy.²² Nevertheless, authors who oppose the routine use of on-site cytological evaluation would agree there is a role for such evaluation in cases where rates of inadequacy are expected to be higher, such as in large cystic masses, multiple large nodules or repeat procedures.²³ Our study demonstrated a higher rate of positive diagnosis with SP-US-FNAC compared to RP-US-FNAC. However, this may have resulted from the small study size.

Another advantage of surgeon-performed ultrasound over a radiology-referral-based ultrasound was illustrated in a study involving breast cancer patients.¹² Rahman et al showed that surgeon-performed ultrasound enabled a diagnosis to be established within 48 hours, which was shorter than radiologist-based ultrasound (median time of 23 days). This resulted in a significant reduction in patient anxiety, which was prevalent in this patient group.^{12,24} Patients also found the surgeon-based system to be more efficient and satisfactory. Our present study on thyroid nodules shows a similar trend in time taken to establish a diagnosis, and corroborates the benefits outlined by Rahman et al. A follow-up study involving patient surveys can be conducted to validate this point.

Another benefit of SP-US-FNAC over RP-US-FNAC is the opportunity for the surgeon to exercise his clinical

judgement using results of both the US and FNAC findings. Al-azawi et al reported two instances in which patients underwent SP-US-FNAC and received a negative result.⁷ Despite that, the surgeon went on to perform thyroidectomy based on suspicious features on the US, and the final pathology confirmed a missed malignancy in both instances. Such scenarios are rare, but they highlight the benefit of having a more streamlined patient management through SP-US-FNAC.

Conclusion

Based on the results of our study, we recommend that head and neck surgeons be trained and routinely perform US-FNAC themselves. This would lead to shorter waiting times and therefore increased patient satisfaction as a result of a more streamlined care and reduction of referrals. Given the benefits of US-FNAC, we also advise that clinicians who are unable to perform US-FNAC should continue to routinely refer their patients to radiologists for US-FNAC of thyroid nodules.

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Chikungunya in Singapore—the Battle Continues

Lynette LE Oon,¹ MBBS, MRCP, FRCPA, Lee Ching Ng,² PhD

In the past decade, Singapore has been confronted by outbreaks caused by novel viruses, namely Nipah virus in 1999, severe acute respiratory syndrome (SARS) coronavirus in 2003, and chikungunya virus in 2008. While Nipah virus and SARS coronavirus disappeared after causing a season of crisis, we continue to see importation of the chikungunya virus after the initial elimination in 2009. In early 2013, chikungunya fever re-emerged in Singapore, culminating in 1059 cases by the end of the year.¹ At the point of writing this commentary, the transmission has subsided.

The chikungunya virus, transmitted by mosquitoes, belongs to the family *Togaviridae* and genus *Alphavirus*. The virus is believed to have originated from Africa, where it is maintained in a sylvatic cycle involving wild primates and forest-dwelling mosquitoes. In urban or rural areas in Asia, chikungunya fever is largely transmitted from human to human via *Aedes aegypti* and *Aedes albopictus*. The disease is characterised by an abrupt onset of high fever, severe arthralgia or arthritis, myalgia, headache and rash. While largely self-limiting, some patients experience joint pain that continues for years.²

Chikungunya virus may have been introduced into Singapore as early as the 1960s but until 2008, there were only sporadic imported cases.^{3,4} Singapore's population remained immunologically naive to the virus as evidenced by the low seroprevalence of 0.4% among 531 young healthy adults tested in 2002 to 2003.⁵ The first reported local transmissions of chikungunya in Singapore occurred in January 2008 in a small outbreak of 13 cases in Little India, a densely populated urban area.³ Despite a successful containment of the virus in Little India in February 2008, the virus emerged again in rural and suburban parts of Singapore in the middle of the year. By 2009 when the outbreak subsided, a total of 1033 chikungunya cases were reported.⁶ Over 75% of the cases were indigenous and 83% of the clusters involved rural or suburban areas.⁴ Between 2010 and 2012, chikungunya fever reverted to

the pre-2008 scenario of mainly being an imported disease, with only 12 indigenous cases and a total of 60 cases, and it appeared that the battle against this virus was won.⁷⁻⁹ However, in 2013, Singapore experienced a resurgence of chikungunya, of which 95% were indigenous cases, mainly in suburban areas in Singapore.¹ Interestingly, the chikungunya epidemic has come on the back of the largest dengue epidemic in Singapore since 2005, suggesting that similar factors may be at play in the resurgence of these two mosquito-borne diseases.

The control of chikungunya is multi-pronged, involving an understanding of the interplay of the mosquito vector, virus, and host factors. The mosquito vector in Singapore's first outbreak in Little India was *Ae. aegypti*, the same urban mosquito vector which drives dengue epidemiology in Singapore.³ In contrast, *Ae. albopictus*, which is mostly associated with greenery, was the predominant vector in the second outbreak in 2008 to 2009 and the current 2013 to 2014 outbreak.³ This explains the higher intensity of transmission in suburban and rural areas of Singapore during these outbreaks. The virus involved in the first outbreak was a wild type virus with alanine at codon 226 of the envelope 1 gene. Virus strains isolated from the latter outbreaks showed the alanine to valine (A226V) mutation, which is known to significantly enhance the transmissibility of the virus by *Ae. albopictus*. The mechanism of the enhancement include the lowering of the oral infectious dose and the increase of viral dissemination in the mosquito.¹⁰

Genetic fingerprints of the viruses detected since 2006 revealed that there have been multiple importations; at least 4 different strains, closely related to strains from India, Malaysia and Sri Lanka, have been associated with outbreaks.³ Insights gained from molecular epidemiology suggested that the 2008 outbreaks were most likely due to importations of the virus from these countries, which were also battling with the disease during the same periods, and subsequent spread to the local naive population.³ Investigation of the 2013 to 2014 outbreak is still unfolding.

¹Department of Pathology, Singapore General Hospital, Singapore

²National Environment Agency of Singapore, Singapore

Address for Correspondence: Dr Lynette Oon, Department of Pathology, Singapore General Hospital, 20 College Road, Singapore 169856.

Email: lynette.oon.l.e@sgh.com.sg

Singapore is not alone in its battle against chikungunya. Since the recent outbreak in Kenya in 2004, the virus has spread globally in a series of epidemics across the Indian Ocean to the Indian subcontinent. It subsequently spread to Southeast Asia and even to southern Europe.¹¹ In December 2013, it arrived at the Caribbean, showing evidence of gaining a foothold in a new territory.¹² Millions of people have been infected in at least 40 countries.¹³ The resurgence in Singapore is facilitated by several factors, and the situation reflects the larger global picture. Firstly, there are vast immunologically naïve local and international populations to a disease that has been rather dormant globally in the last few decades. Secondly, an increase in international travel has allowed the cross-border spread of the disease, and local travel and migration have facilitated further spread within countries, including to rural isolated areas. Thirdly, the genetic adaptation of the virus to *Ae. albopictus* has contributed to the waves of epidemics across the globe.¹⁰ *Ae. albopictus*, originally native to Asia, has greatly expanded its geographic range in the last two decades to include Africa, the Americas, Europe and the Middle East, spanning tropical and temperature latitudes.¹⁴ Fourthly, rising population density in countries such as Singapore provides abundant opportunities for interactions between humans and the mosquito vector. Lastly, environmental factors such as temperature and rainfall probably play an important role in chikungunya transmission.¹¹ It is interesting that chikungunya outbreaks are driven by different environmental factors in different settings. In Singapore, peak chikungunya outbreaks are associated with rainy seasons, while reports have described that the outbreak in Kenya in 2004 was associated with drought conditions.¹⁵

Vector control is the sole method for reducing transmission of chikungunya as no vaccine is available. Having *Ae. albopictus* as the main mosquito vector for the chikungunya virus presents additional challenges to vector control services already over-taxed by the dengue epidemic. In contrast to *Ae. aegypti* which mainly inhabits urban areas, *Ae. albopictus* is largely outdoors, so measures such as outdoor fogging, residual spraying of external walls and clearing of unkempt vegetation will have to go hand-in-hand with current vector control strategies aimed at reducing dengue transmission.¹⁶ *Ae. albopictus* is relatively long-lived (4 to 8 weeks), has a flight radius of 400 m to 600 m and its eggs are highly resistant to drying. Its association with nature and greenery may render its population even more difficult to reduce and eliminate as compared to *Ae. aegypti*, which is already a challenge both locally and worldwide. In the Hawaiian islands, an extensive mosquito control campaign virtually eliminated *Ae. aegypti*, thus controlling their dengue epidemic in 1943 to 1944, but *Ae. albopictus* remained widespread.¹⁷

The chikungunya epidemiology clearly demonstrates the vulnerability of the globalised and immunologically naïve human population to diseases vectored by mosquitoes that share the same ecological niche as the human population. In Singapore, these characteristics of chikungunya epidemiology mirror that of dengue.¹⁸ Though the extensive national vector control programme may have moderated the impact of these diseases, the increasing challenges call for novel approaches to augment the effectiveness of the programme in tackling these diseases. This includes the use of new technology such as population replacement or suppression by the *Wolbachia*-infected *Aedes* or genetically modified sterile male *Aedes*.

The battle against chikungunya involves an understanding of the complexity of vector-virus-host-environment interactions and how they can affect disease epidemiology. In particular, the study of virus evolution is key to understanding virus adaptation to the vector and subsequent transmission. There is a continued need for intense surveillance, improved diagnostics, and rigorous research of this virus in order to succeed in the battle.

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Implications of Morbid Obesity and Surgery for the Obese

Lynette MA Loo, ¹MBBS, MRCS, MPH, Asim Shabbir, ¹MBBS, MMed, FRCS, Jimmy BY So, ¹MBChB, FRCSEd, MPH

Since the dawn of mankind, the survival of the species theory mandates the adaptation of complex mammalian bodies to harsh climates and unfavourable environments. With abundant nutrition, sedentary lifestyle and modern transportation amongst many other comforts of urbanisation, many of the overweight and obese men today would be unrecognisable from the common man in the days of Socrates, Leonardo Da Vinci and even Thomas Edison.

Modern healthcare facilities are plagued with diseases of the metabolic syndrome as the rate of obesity in Singapore and most developed countries rises unchecked. This is now a global public health problem, a pandemic plaguing the rich and the poor, the young and the old, the movers and shakers and even the downtrodden. According to the Ministry of Health, Singapore, the overall prevalence of obesity in 2010 is 11%, with 1.7 million of Singaporeans being at risk of obesity-related health problems.¹

Body mass index (BMI) is commonly used to objectively measure the status of weight. BMI is calculated as weight in kilograms divided by the height in meters squared (m^2). According to the WHO classification of overweight and obese in Asian countries, an overweight individual has a BMI between 23 and 27.5 kg/m^2 . In Singapore and Asia-Pacific countries, an obese person is defined as a person with BMI of 27.5 kg/m^2 or more.² Obesity is classified by WHO as a chronic disease, a multisystemic disease resulting from complex interaction between the human genotype and the environment. Morbid obesity is defined as patients whose weight is more than twice their ideal body weight and/or 100 pounds or more overweight.

As part of the 1998 National Health Survey in Singapore, a study by Deurenberg-Yap et al from the Ministry of Health was published. It showed that for the same amount of body fat as Caucasians, the BMI in Chinese, Malays or Indians would be lower. For example, the BMI cut-off points for obesity would have to be about 27 kg/m^2 for Chinese and Malays and 26 kg/m^2 for Indians compared to the BMI of 30 kg/m^2 in Caucasians (cut-off for obesity as defined by WHO). This is the paradox of low BMI and high body fat percentage among Chinese, Malays and Indians in Singapore.³

Morbid obesity may affect every organ system such as the cardiovascular, respiratory, metabolic, musculoskeletal, gastrointestinal, urological, endocrine and reproductive, dermatological, neurological and psychological systems, thus increasing the risks of developing many diseases. High BMI is on average associated with about 30% higher overall mortality — 40% increased mortality rate for vascular mortality, 60% to 120% for diabetic, renal, and hepatic mortality, 10% for neoplastic mortality and 20% for respiratory and for all other mortality.⁴

Various comorbid conditions coexist or are common in the obese such as:

- hypertension, atherosclerotic heart and peripheral vascular disease with myocardial infarction and cerebral vascular accidents, peripheral venous insufficiency, thrombophlebitis, pulmonary embolism
- asthma, obstructive sleep apnea, obesity-hypoventilation syndrome
- type 2 diabetes, impaired glucose tolerance, dyslipidemia
- cholelithiasis, gastroesophageal reflux disease
- non-alcoholic fatty liver disease or steatohepatitis, hepatic cirrhosis
- hepatic carcinoma, colorectal carcinoma, cancer of the endometrium, breast, ovary, prostate, pancreas
- back strain, disc disease, osteoarthritis of the hips, knees, ankles, feet
- polycystic ovary syndrome, increased risk of pregnancy and foetal abnormalities, male hypogonadism
- intertriginous dermatitis
- pseudotumour cerebri
- carpal tunnel syndrome
- depression, eating disorders, body image disturbance.

Without medical intervention, average efficacy of a very strict, rigorous but unsupervised weight-loss programme results in weight loss of about 20 kg per year. Commercial

¹University Surgical Cluster, National University Hospital, Singapore

Address for Correspondence: A/Prof So Bok Yan, University Surgical Cluster, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074.

Email: jimmy_so@nuhs.edu.sg

options cost billions of dollars worldwide and the results are often times transient and unpredictable. In the past, most prescription pharmacological agents are not licensed for bariatric patients and could not be used in this population of patients.⁵

Numerous pharmaceutical giants are running trials on various novel agents in the field of weight management. Orlistat, a pancreatic lipase inhibitor, could result in weight loss of 3 to 4 kg per year in addition to diet and exercise programmes. However, they may cause significant side effects such as diarrhoea, cramping, flatulence and steatorrhoea.⁵

Other upcoming medications include Contrave, Empatic™, Qnexa, and an injectable combination of leptin and pramlintide. The only United States (US) Food and Drug Administration (FDA)-approved medication is Qnexa which is a combination of phentermine and topiramate. Contrave is a combination of bupropion and naltrexone and it is in Phase 3 clinical trial to prove its cardiovascular safety profile. Meanwhile the Phase 2b trial for Empatic™ has just been concluded and it is a combination of bupropion and zonisamide, awaiting start of Phase 3 trial.⁶

Bariatric surgery remains the cornerstone of the management of obesity, with proven safety, efficacy and durability.⁷⁻⁹ The latest consensus statement from the American Society for Bariatric Surgery and the American Society for Bariatric Surgery Foundation published in 2005 concluded that bariatric surgery is the most effective treatment available for complete resolution or improvement in co-morbidities associated with obesity.⁶

Critical events since the American 1991 NIH Consensus Conference included the rapid advancements in laparoscopic bariatric surgery with acceptable morbidity, mortality and long-term complications, increased experience with multi-disciplinary team-based management, increasing numbers of bariatric surgery in the adolescent and elderly and cost-effectiveness.⁶

Most importantly, data has now been established in randomised controlled trials comparing bariatric surgery and intensive medical therapy. For example, the Cleveland Clinic group has reported improvements in co-morbidities related to obesity including reversal or improvement in diabetes mellitus. This is especially with regards to mean levels of glycated haemoglobin, dyslipidemia, hypertension, metabolic syndrome with significant reduction in the use of diabetic and hypertensive medications.⁹

Selection of candidates for bariatric surgery starts with meeting the NIH Consensus guidelines including weight criteria. In Asian countries, this includes BMI of more than 37 kg/m² in those without concomitant co-morbid diseases or BMI of more than 32 kg/m² in the presence of severe comorbidities.¹⁰

High-risk co-morbid conditions that can justify reducing the BMI to 32.5 kg/m² include type 2 diabetes, life-threatening cardiopulmonary problems (eg. severe sleep apnea, obesity-related cardiomyopathy, etc), obesity-induced physical problems interfering with a normal lifestyle and body size problems severely interfering with employment, family function, and ambulation. These patients are also screened for co-existing mental disorders that preclude compliance to treatment and lifestyle advice post-surgery.

Various surgical options available in all major hospitals in Singapore include sleeve gastrectomy, Roux-en-Y gastric bypass, gastric banding and biliopancreatic diversion with duodenal switch operation.¹¹⁻¹⁴ Indications, risks and benefit ratio and patients' preference would be considered in making the final decision on the best surgical approach for the individual patient during each consultation with the weight management team. This multi-disciplinary team usually consists of bariatric surgeons, bariatric specialist nurses, dieticians, physiotherapists and even counsellors.

Therefore, intense public health momentum on preventive measures, together with current advances in weight management and bariatric surgery offer realistic hope of remission in many medical conditions in obese patients.

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Haemoglobin E-beta Thalassaemia in Singapore

Dear Editor,

Haemoglobin E-beta thalassaemia (Hb E/ β -thalassaemia) is a common cause of thalassaemia major in Southeast Asia. In Singapore, the prevalence of beta thalassaemia (β -thalassaemia) trait is 0.9% and haemoglobin E (HbE) trait is 0.55%.¹ This is especially high in the Malay ethnic group. According to the National Thalassaemia Registry, the Malay population accounts for 74% of the registered subjects with Hb E/ β -thalassaemia.

The clinical and haematological parameters of patients with Hb E/ β -thalassaemia are widely disparate, ranging from asymptomatic to severe, life-threatening anemia requiring regular blood transfusions. Its phenotypic variability makes it difficult to predict the course of the disease, especially in the prenatal setting.

Several factors have been suggested to influence the severity of disease. Genetic factors include the genotype of β -thalassaemia,² the presence of concomitant alpha thalassaemia (α -thalassemia) and the presence of polymorphism associated with increased foetal haemoglobin (HbF). Environmental influences such as the presence of anti-malarial antibodies have also been implicated.³

The aim of this study is to conduct a retrospective case analysis of Hb E/ β -thalassaemia cases in our local population.

Materials and Methods

Study Population

The study population includes patients with Hb E/ β -thalassaemia referred to the National Thalassaemia Registry in Singapore between 2000 and 2011 and who have been reviewed by a clinician in KK Women's and Children's Hospital.

Data Collection

Records of all patients were reviewed to obtain the following information: demographic data, transfusion and chelation history and history of medical complications. The general surveillance of iron overload in our hospital includes measurement of ferritin, renal and liver function, calcium and phosphate levels, thyroid function test, fasting glucose measurements and cardiac magnetic resonance imaging

(MRIT2*). Mean haemoglobin levels, HbF levels, patient's genotype and the presence of concomitant α -thalassemia were also recorded. Severity score for each patient was calculated using the scoring system by Sripichai et al.⁴ Approval was obtained from the institutional review board.

Laboratory Methods

Hb electrophoresis was carried out on the Bio-Rad Variant II using the β -thalassaemia short (BTS) programme. β -thalassaemia mutation was determined by screening through 48 mutations reported in the Asian regions using reverse dot blot. For patients with raised HbA₂ but negative for the above mutation, sequencing of β -globin gene was done.

Results

Demographics

Fifty-eight subjects were identified. Patient records were incomplete for 3 patients and 55 patients were recruited into the study. There were 26 females and 29 males. Median age was 13 years (range, 3 to 54 years) at the time of enrollment. The study population consists of 43 Malay, 11 Chinese and 1 Thai patient.

Transfusion and Chelation History

Twenty patients (40%) presented below 3 years old, 26 (47%) presented between the ages of 3 to 10 years old and 7 (13%) presented after 10 years old. Twenty-seven patients were transfusion dependent thalassaemias, with 24 on chelation therapy.

Medical History

Twenty patients (36%) had a splenectomy. Fourteen (25%) patients had developed gallstones and 7 had a cholecystectomy performed. Other medical complications of disease and iron overload are summarised in Table 1.

In addition, 21 out of the 55 patients had a MRI T2* of the heart and liver performed. None had iron loading in the heart. Twenty (98%) patients had evidence of iron loading in the liver. Out of the 20 patients with iron loading in the liver, 7 did not have regular blood transfusions.

Table 1. Medical Complications in Patients (n = 55)

Complication	No. of patients (%)
Hypothyroidism	4 (7.2%)
Hyperparathyroidism	0
Short stature	22 (40%)
Delayed puberty	6 (11%)
Diabetes mellitus	0
Osteoporosis	3 (5.4%)
Pathological fracture	1 (1.8%)
Leg ulcer	1 (1.8%)

Severity Score

The patients were divided into 3 groups according to their severity score. A total of 21 patients belonged to the mild category, 19 to the moderate category and 15 to the severe category.

Mild Category

The mean haemoglobin (Hb) was 8.3 g/dL (range, 6.9 to 10.7 g/dL; SD 1.0). Only one of the patients required occasional transfusions. The mean foetal haemoglobin (HbF) was 35.6% (range, 6.1% to 56.5%; SD 16.1). Two patients underwent splenectomy. MRI T2* imaging was performed for 3 patients. None had cardiac iron overload, but all three had moderate iron load in the liver.

Moderate Category

The mean Hb was 7.4g/dL (range, 6.5 to 9.8 7.4g/dL; SD 1.1) with HbF 39.1% (range, 9.2% to 60.2%; SD 15.2). Two patients had concomitant α -thalassemia.

Thirteen (68%) patients in this category required regular transfusions. Seven required splenectomy. MRI T2* was performed for 8 patients in this category with 4 showing mild, 3 moderate and 1 severe iron loading of the liver.

Severe Category

There were 15 patients in this category. The mean Hb was 6.3 g/dL (range, 5.2 to 7.5 g/dL; SD 0.7). The mean HbF was 44.7% (range, 32% to 64.6%; SD 11.8). One patient had concomitant α -thalassemia. Two patients had passed away at age 13 and 27. Fourteen (93%) patients were transfusion dependent.

This group of patients had the most complications, accounting for 5 of the 6 patients with delayed puberty and all of the patients with hypothyroidism. Eleven patients had a splenectomy at an average age of 7.6 years. MRI T2* imaging was performed for 10 of these patients with 3 showing mild iron loading of the liver, 4 moderate and 2 severe.

Genotyping of Hb E/ β -thalassaemia was performed in 47 patients and is presented in Table 2.

Table 2. β -Thalassaemia Mutations of Patients (n = 47)

	No. of patients in mild category	No. of patients in moderate category	No. of patients in severe category
IVS-1 nt 5 (G/C)	5	7	5
IVS-1 nt 1 (G/T)	1	0	1
Codon 35 (-C)	1	1	2
CDs 41/42 (-TTCT)	1	1	3
Codon 19 (A/G)	2	0	0
-28 TATA box (A/G)	2	0	0
Others	4	6	5
Total no. of patients with genotyping performed	16	15	16

β : Beta

Discussion

Thalassaemia is the most common autosomal recessive condition in Singapore. The phenotypic heterogeneity of Hb E/ β -thalassaemia remains a challenge. In our study cohort, 49% of patients with Hb E/ β -thalassaemia required regular blood transfusions and most of these patients were on chelation therapy. In previous studies, the percentage of patients requiring regular transfusion ranged from 30% to 57%.⁵ Also in our study, 36% of patients required splenectomy. In other case series, the rate of splenectomy ranged from 17% to 50%.

Our patients also had significant medical complications, the most common of which is iron loading of the liver. It is important to note that 35% of these patients did not have regular blood transfusions. This highlights the need to monitor the patients for iron overload regardless of their transfusion status.

When we divided the patients into three different disease severities, there was no clear factor that significantly distinguished the 3 groups. Patients with β^0 mutations, such as CDs 41/42 (-TTCT), CD 35 (-C), CD 17 (A > T), 45 kb Filipino deletion, tend to have higher risk of severe phenotype compared with those with β^+ mutations such as those in IVS-1 and TATA box region, although this correlation is not absolute. As expected, complications of iron overload were most evident in the severe category.

Our study is limited by our small sample population. Another limitation of our study is the potential bias as milder patients may be asymptomatic and therefore not be evaluated in our hospital.

Conclusion

The phenotypic variability of Hb E/ β -thalassaemia presents a challenge in counselling and management. In our population, a significant proportion of patients with Hb E/ β -thalassaemia have severe disease requiring regular blood transfusions and chelation therapy. There is also a need to closely monitor all patients regardless of their transfusion status.

Ee Shien Tan, ¹MBBS, MRCPCH, Cedric Koh, ²MBBS, Hai Yang Law, ³DPhil, Guek Peng Tan, ³BSc, Angeline Hwei Meeng Lai, ¹MBBS, MRCP, Ivy Swee Lian Ng, ¹MBBS, MMED, FRCP

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¹Genetics Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore

²Yong Loo Lin School of Medicine, National University of Singapore, Singapore

³DNA Diagnostic and Research Laboratory, KK Women's and Children's Hospital, Singapore

Address for Correspondence: Dr Ee Shien Tan, Genetics Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899.
Email: Tan.Ee.Shien@kkh.com.sg

Treatment-related Acute Myeloid Leukaemia After Temozolomide for Glioblastoma Multiforme

Dear Editor,

Temozolomide (TMZ) is an orally administered analogue of dacarbazine and is considered as a second-generation alkylating agent with antineoplastic activity. It exerts its effect primarily via deoxyribonucleic acid (DNA) methylation at the O6 and N7 positions of guanine leading to DNA replication inhibition. It received accelerated approval by the United States (US) Food and Drug Administration (FDA) in 1999 for the treatment of refractory anaplastic astrocytoma in adults. Subsequently it has been approved by the FDA in 2005 for treatment of newly diagnosed glioblastoma multiforme (GBM), initially in combination with radiotherapy, then as maintenance treatment.¹ In Singapore, TMZ has been approved for use by the Health Sciences Authority (HSA) since November 2001.

Under World Health Organization's (WHO) classification, there are 2 main types of therapy-related myeloid neoplasms (t-MNs): alkylating agent/radiation-related and topoisomerase II inhibitor-related acute myeloid leukaemia (AML).² These conditions arise following the use of cytotoxic chemotherapy and/or radiation therapy. For the alkylating agent-related ones, it frequently presents as myelodysplastic syndrome (MDS) initially. It may then evolve into AML. This group of secondary MDS and AML commonly has complex cytogenetics and unbalanced translocations or deletions involving primarily chromosomes 5 and/or 7.

There were a few reports of TMZ-associated MDS/AML and all of these patients described had either received other cytotoxic agents in addition to TMZ and radiation, or their AMLs were preceded by a period of marrow failure in the form of MDS.³⁻⁸ We present a case of AML at initial presentation following the use of TMZ and radiation for GBM.

Case Report

The patient was a 56-year-old Chinese female who was diagnosed with GBM (WHO grade 4) involving the left occipital and insular regions at the end of January 2008 after complaining of headache. The diagnosis was based on brain biopsy performed. She had no significant past medical history of note and no family history of malignancy. She was initially given dexamethasone. She subsequently

underwent intensity-modulated radiation therapy of 60 Gy for 30 fractions from 27 February 2008 to 9 April 2008. She was given TMZ 75 mg/m²/day concurrently with the radiation therapy. Subsequently, she was given TMZ 150 mg/m²/day for 5 days every month from 20 May 2008. She received a total of 8 cycles of TMZ and her last dose was from 22 December 2008 to 26 December 2008. She was also on tapering dose of dexamethasone till September 2008. Her full blood counts prior to initiation of TMZ were unremarkable with haemoglobin of 13.0 g/dL, white blood cell (WBC) of 8.6 x 10⁹/L and platelet of 219 x 10⁹ L.

Magnetic resonance imaging (MRI) brain assessment of the GBM conducted in early November 2008 demonstrated partial response with decrease in size of the occipital lesion while the insular lesion was stable. She tolerated TMZ well and only experienced grade 2 haematologic toxicity affecting solely the haemoglobin level (haemoglobin was 9.5 g/dL) (common toxicity criteria) since early December 2008.

She first developed grade 3 haematological toxicity (common toxicity criteria) on 13 January 2009. Her full blood counts then showed haemoglobin of 6.3 g/dL, WBC count of 1.9 x 10⁹/L (with absolute neutrophil count of 0.83 x 10⁹/L) and platelet of 70 x 10⁹ L. Initial impression was likely TMZ-related myelosuppression and her upcoming cycle of TMZ was held off. TMZ was stopped since then. Her counts continued to be low, requiring weekly blood and platelet transfusions. She also received 2 doses of filgrastim for her severe neutropenia on 20 and 21 January 2009. Her full blood counts on 29 January 2009 showed haemoglobin of 8.2 g/dL, WBC count of 1.52 x 10⁹/L (neutrophil count of 0.47 x 10⁹/L) and platelet of 36 x 10⁹ L. In view of such severe and prolonged cytopenias, referral to the haematology department was made. Bone marrow evaluation (including bone marrow aspirate and trephine, and tests for flow cytometry and karyotype profile) was conducted on 30 January 2009. In summary, there were features compatible with AML (blast cells of 23% demonstrated in bone marrow aspirate) with background of myelodysplastic syndrome (Fig. 1) and complex cytogenetics (Fig. 2).

The patient received induction chemotherapy that included 3 days of idarubicin (12 mg/m²/day by slow bolus on days 1, 2 and 3) and 7 days of cytarabine (100 mg/m²/day by continuous infusion) (3 + 7) in February 2009.

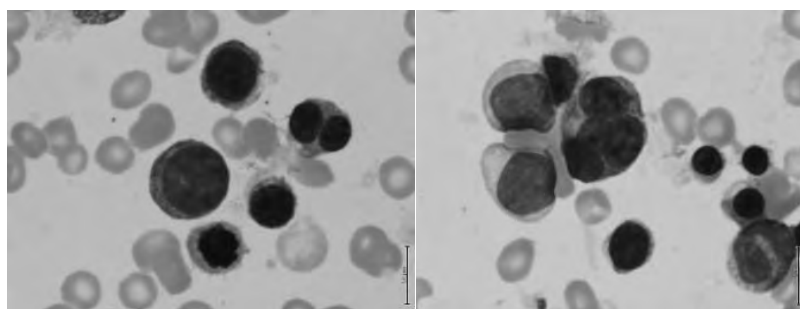


Fig. 1. Two bone marrow aspirate slides of the patient demonstrating myeloid blasts and dyserythropoiesis. (May-Grunwald-Giemsa stain, x 1000).

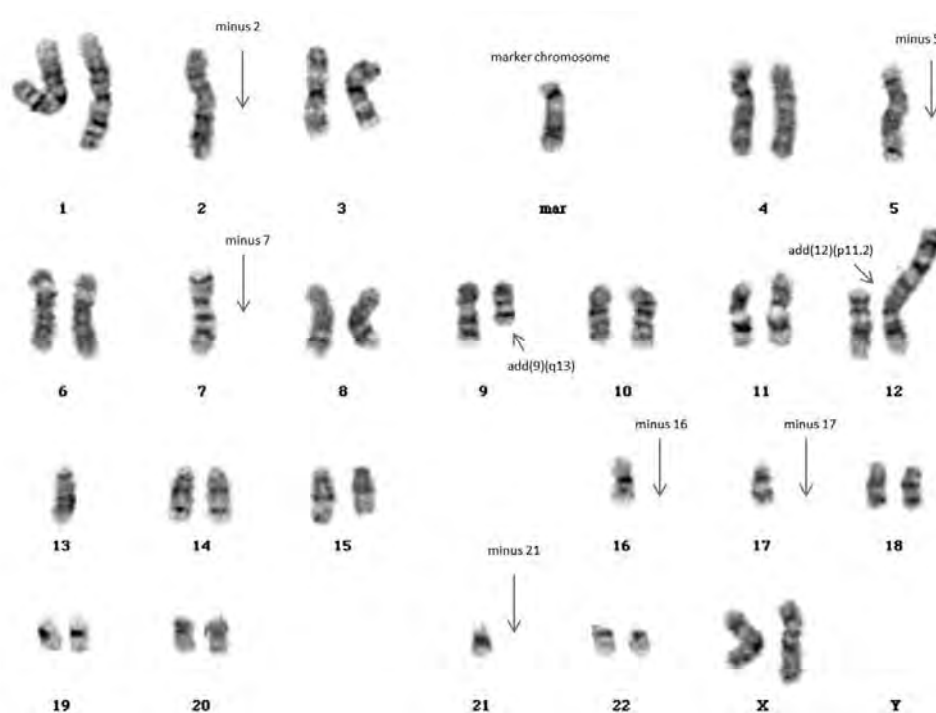


Fig. 2. Karyogram of the patient demonstrating a hypodiploid cell with numerical and structural abnormalities, specifically monosomies 5 and 7 were observed. (NB: Cytogenetic analysis was done on bone marrow cells directly after 40 minutes of incubation in a colcemid-containing hypotonic solution, and after short-term cultivation for 24 hours using RPMI 1640 culture medium. Twenty cells were analysed, chromosomal abnormalities were described according to ISCN 2009.)

Post-induction bone marrow evaluation revealed persistence of approximately 5% of blasts and she received a further cycle of 2 days of idarubicin (12 mg/m²/day by slow bolus on days 1 and 2) and 5 days of cytarabine (100 mg/m²/day by continuous infusion) (2 + 5) in March 2009. This was initially meant to be consolidation chemotherapy but the cytarabine was shortened from 7 to 5 days when her GBM showed progression on MRI. No further intensive chemotherapy was given after that as her cytopenia had resolved. With the treatment intent of her AML switched to palliative due to limited treatment options available for her progressive GBM, no further bone marrow investigations were done to confirm her response to the second cycle of idarubicin and cytarabine.

However, her cytopenia recurred at the end of June 2009 and was deemed to be due to relapse of her AML. She received 1 cycle of decitabine (20 mg/m² for 5 days) in

mid-August 2009 with little haematological response. In mid-September 2009, she developed headache with new onset of expressive dysphasia. Computed tomography (CT) brain demonstrated several new foci of lesions suspicious of intratumoral haemorrhage. Patient was given blood product support and palliative treatment. She passed away at the end of September 2009.

Discussion

Prior to the use of TMZ, nitrosoureas have been widely used for the treatment of gliomas. However, nitrosoureas is more myelotoxic than TMZ and prolonged use of nitrosoureas is not feasible given the progressive significant myelosuppression and toxicity that will ensue. TMZ, on the other hand, is much well tolerated. Concurrent radiation therapy with TMZ treatment for glioblastoma resulted in grade 3 or 4 haematological toxicity in approximately 7%

of patients only.¹ This opens up the possibility of prolonged use of TMZ in patients with gliomas who have responded to its treatment. Having established that, treatment of gliomas with TMZ is still non-curative, similar to nitrosoureas.

Mustafa et al reported a small case series of prolonged TMZ use of at least 5 years with minimal long-term toxicity.⁹ There are also reports of severe haematological toxicities such as pancytopenia, t-MDS and leukaemias following the use of TMZ.³⁻⁸ The time of exposure prior to the development of such toxicities can be as short as weeks. However, most of the patients described had been exposed to the other alkylating agents, of which the contribution to leukemogenesis cannot be excluded. The only form of chemotherapy that our patient received prior to her AML diagnosis was TMZ and hence this provides the most direct evidence of the role of TMZ in the development of secondary AML. All the AML cases described so far evolved from MDS (majority of which took a few months and only one reported progression to AML 1 week after diagnosis of MDS). Our patient presented with AML (WHO 2008 definition)² at the point of diagnosis which is unusual. We could not verify from past published reports if this is attributed to the revised WHO 2008 definition of AML which classifies all myeloid neoplasms with at least 20% as AML, rather than 30% in the past. The duration of the first onset of significant cytopenias to the evaluation of her bone marrow was only slightly more than 2 weeks. The presence of highly complex cytogenetic abnormalities that include deletion of chromosomes 5 and 7 in our patient were suggestive of alkylating agent/radiation-related AML.

While clinically significant haematological toxicity is not common with TMZ as first described by Stupp et al,¹ such toxicity may potentially emerge as an important side effect as it is now a common practice for many physicians to extend the treatment of TMZ for patients with non-progressive disease. This extension may be up to 12 to 36 months, much longer than the duration of TMZ exposure described originally (up to 628-day cycle of adjuvant TMZ). Apart from a phase 2 trial on continuous use of TMZ for recurrent malignant gliomas that demonstrated severe haematological toxicity was uncommon,¹⁰ data regarding the efficacy and toxicity of protracted course of TMZ are, however, lacking. The optimal duration of TMZ therapy for patients who have good response has yet to be established. Our patient's GBM was responding to TMZ and her treatment was extended to 8 cycles. Unfortunately the development of therapy-related AML (t-AML) necessitated the cessation of TMZ. The progression of the GBM after 3 months of stopping TMZ subsequently became the limiting factor for further aggressive treatment of her t-AML. Given that such toxicity may be life threatening to those at risk, reports of clinical and genetic factors in predicting this risk would be worth exploring further.

Till the end of April 2013, there had been 383 patients who were treated with TMZ in our institution. To the authors' best knowledge, this was the only case of TMZ-associated MDS/AML. Although the true incidence of TMZ-related MDS/AML could not be calculated based on these data, it seems the occurrence of such complication is uncommon. Therefore, TMZ is still likely the most suitable treatment option for patients with GBM. The paucity of TMZ-associated MDS/AML may be partly due to the poor prognosis of patients with malignant gliomas.

Optimal treatment for patients with t-MDS/AML has not been established and many factors including the patient's age, performance status, concurrent medical conditions and status of the primary malignancy play a pivotal role in deciding the management approaches for these patients. Intensive chemotherapy followed by allogenic stem cell transplantation may be the only curative option for eligible patients but is often not a suitable option for patients with malignant gliomas due to the poor prognosis of the latter. t-MDS/AML also confer an unfavourable prognosis which often cannot be cured by intensive chemotherapy alone and is associated with a median overall survival of only 6 months.^{11,12}

Conclusion

Clinicians should be aware of the potentially serious haematological side effects of t-MDS/AML with the use of TMZ. Although this adverse effect is rare and is outweighed by the benefits of TMZ, t-MDS/AML may worsen the overall prognosis of the patient and may occur after only short courses of TMZ.

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Chuen Wen Tan, ¹MB BCh BAO, MRCP (UK), Siew Ju See, ²MBBS, MRCP (UK), Chee Kian Tham, ³MBBS, MRCP, Ai Leen Ang, ¹MBBS, FRCPath (UK)

¹Department of Haematology, Singapore General Hospital, Singapore
²Department of Neurology (SGH Campus), National Neuroscience Institute, Singapore
³Department of Oncology, National Cancer Centre Singapore, Singapore

Address for Correspondence: Dr Chuen Wen Tan, Department of Haematology, Singapore General Hospital, Outram Road, Singapore 169608.
 Email: tan.chuen.wen@sgh.com.sg

A Rash in an Elderly Woman

An 82-year-old Chinese woman presented with a 1-week history of an increasingly pruritic rash over the face, neck, upper chest and arms. She was first seen by a haematologist 3 months prior for acute monocytic leukaemia (AML-M5) which had developed on a background of probable chronic myelomonocytic leukaemia (CMML). As she had multiple comorbidities including previous stroke with limited ambulatory function, she was considered a poor candidate for intensive chemotherapy and was treated palliatively with supportive blood transfusions when needed. Her medications included omeprazole, lactulose, senna, paracetamol, calcium, vitamin D, risedronate, and a recent course of co-trimoxazole for an upper respiratory tract infection (commenced 7 days prior to presentation). She received her most recent blood transfusion one month prior to onset of the rash.

Examination revealed multiple purpuric papules and plaques distributed on the cheeks (Fig. 1), neck, upper chest (Fig. 2) and upper limbs. The patient was afebrile and non-toxic. Skin biopsies were obtained from a representative forearm lesion.

What is your diagnosis?

- A. Drug eruption
- B. Acne rosacea
- C. Leukaemia cutis (cutaneous leukaemic infiltrates)
- D. Acute febrile neutrophilic dermatosis (Sweet syndrome)
- E. Cutaneous vasculitis



Fig. 1. Purpuric papules and plaques on the face.



Fig. 2. Purpuric papules and plaques on the neck and upper chest.

Discussion

Histopathological examination revealed superficial and deep perivascular and patchy upper dermal interstitial infiltrate of lymphocytes which formed tight cuffs around upper dermal vessels, accompanied by prominent red cell extravasation within the dermis. Blast cells with large, rounded to hyposegmented nuclei with dispersed chromatin, and variable amounts of cytoplasm were scattered within the infiltrate (Fig. 3). These stained strongly positive for myeloperoxidase and CD117, were patchily positive for CD34 and CD68, and negative for CD3 and CD20. This confirmed the diagnosis of leukaemia cutis (LC). Direct immunofluorescence was negative. Her full blood count showed marked thrombocytopenia ($8 \times 10^9/L$), anaemia (haemoglobin 8 g/dL) and mild leukocytosis (total white cell count $13.3 \times 10^9/L$) with increased blasts (16%).

The dual burdens of severe pruritus and cosmetic distress warranted intervention. After discussion with the patient and her family, a course of palliative chemotherapy with etoposide was given. Itch and number of skin lesions were significantly reduced, along with decrease in the number of blasts in peripheral blood. Her symptoms were well-palliated up to 2 months after chemotherapy when she was last seen for her skin lesions. She finally succumbed to the disease after another 6 months.

LC refers to cutaneous infiltration by neoplastic leukaemic cells resulting in clinically evident skin lesions, and is a rare condition that can occur across various forms of leukaemia.¹

Answer: C

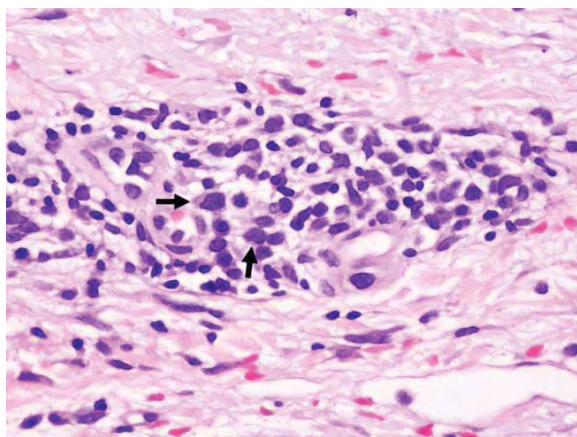


Fig. 3. Within the perivascular infiltrate are blast cells with large rounded to hyposegmented nuclei, dispersed chromatin, and variable amounts of cytoplasm (block arrows). Typical lymphocytes are scattered within the infiltrate (H&E stain, x 400).

It has been described to occur mostly in acute myeloid leukaemia (AML), but also occurs in the accelerated phase of chronic myeloid leukaemia (CML), myelodysplastic syndrome (MDS), and more rarely, in acute lymphocytic leukaemia (ALL). Its appearance often signals underlying disease progression, or transformation to acute leukaemia in the setting of MDS. There are also rare cases of ‘aleukaemic leukaemia cutis’ described in which cutaneous infiltrations preceded peripheral blood or bone marrow involvement.²

Cutaneous manifestations of LC are non-specific and variable, ranging from papules and plaques, purpura, exfoliative erythroderma and urticaria, to non-specific exanthema, and may manifest in several different forms over the course of the disease.¹ The most common manifestation is with single or multiple erythematous papules and nodules, most commonly involving the lower limbs, upper limbs, trunk and face, with a predilection for sites of previous or active inflammation.¹

The diagnosis of LC can be challenging, as LC can be both clinically and histologically confused with other skin conditions. In particular, Sweet syndrome (acute febrile neutrophilic dermatosis) must be excluded. Sweet syndrome is a reactive skin condition occasionally associated with AML, in which intense infiltration of the dermis with neutrophils results in oedematous tender cutaneous papules and nodules associated with fever. Drug eruptions and reactive vasculitis are also not uncommon in patients on treatment for malignancy. Careful clinical examination and skin biopsy are essential for excluding these differential diagnoses, of which the management and prognosis are vastly different from LC.

Skin biopsy in LC typically shows an interstitial infiltrate of leukaemic cells in the dermis and/or subcutis,

exhibiting perivascular and/or periadnexal arrangements, with the presence of immature malignant cells which characteristically appear large, with smooth chromatin, increased mitotic activity, and a high nuclear to cytoplasmic ratio.³ Further immunohistochemical studies can help confirm diagnosis and aid cell lineage profiling.

The diagnosis of leukaemia cutis is important for several reasons. It can occasionally precede and aid in the early diagnosis of systemic leukaemia (as in aleukaemic leukaemia cutis) and is often an indicator of disease progression in established MDS or leukaemia. LC also prognosticates poor outcome as CMML patients with LC have reduced survival time compared to those without LC.⁴ Occasionally, if LC is symptomatic, it can warrant more aggressive treatment in patients on supportive treatment, as seen in this case.

Treatment of LC predominantly involves management of the underlying malignancy. Chemotherapy regimen choice and dosing should follow standard age-, cytogenetic- and molecular-based risk profiling.¹ Additional consideration should be given for haematopoietic cell transplantation or total skin electron beam therapy after chemotherapy for persistent LC if marrow negative.¹ Local treatment in the form of electron beam therapy or radiation therapy for palliation of pain or pruritus may be helpful in patients who are not candidates for chemotherapy. This patient was treated with chemotherapy aimed at symptomatic relief of LC.

In summary, we present a rare case of LC with florid clinical signs requiring cooperative management between dermatologist and haematologist for best patient care. Diagnosis of LC is crucial as it significantly influences patient management.

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Yee Kiat Heng, ¹*MBBS, MRCP (UK), M Med (Int Med)*, Beatrice SS Huang, ²,
Lian King Lee, ³*MB ChB, MRCP, FRCPath*, Joyce SS Lee, ¹*MBBS, MRCP*
(UK), *M Med (Int Med)*, See Ket Ng, ¹*MBBS, M Med (Int Med)*

¹Department of Dermatology, National Skin Centre, Singapore

²Faculty of Medicine, University of New South Wales, Sydney, Australia

³Department of Haematology, Tan Tock Seng Hospital, Singapore

Address for Correspondence: Dr Yee Kiat Heng, Department of Dermatology,
National Skin Centre, 1 Mandalay Road, Singapore 308205.
Email: ykheng@nsc.gov.sg

A Rash in an Elderly Woman

An 82-year-old Chinese woman presented with a 1-week history of an increasingly pruritic rash over the face, neck, upper chest and arms. She was first seen by a haematologist 3 months prior for acute monocytic leukaemia (AML-M5) which had developed on a background of probable chronic myelomonocytic leukaemia (CMML). As she had multiple comorbidities including previous stroke with limited ambulatory function, she was considered a poor candidate for intensive chemotherapy and was treated palliatively with supportive blood transfusions when needed. Her medications included omeprazole, lactulose, senna, paracetamol, calcium, vitamin D, risedronate, and a recent course of co-trimoxazole for an upper respiratory tract infection (commenced 7 days prior to presentation). She received her most recent blood transfusion one month prior to onset of the rash.

Examination revealed multiple purpuric papules and plaques distributed on the cheeks (Fig. 1), neck, upper chest (Fig. 2) and upper limbs. The patient was afebrile and non-toxic. Skin biopsies were obtained from a representative forearm lesion.

What is your diagnosis?

- A. Drug eruption
- B. Acne rosacea
- C. Leukaemia cutis (cutaneous leukaemic infiltrates)
- D. Acute febrile neutrophilic dermatosis (Sweet syndrome)
- E. Cutaneous vasculitis

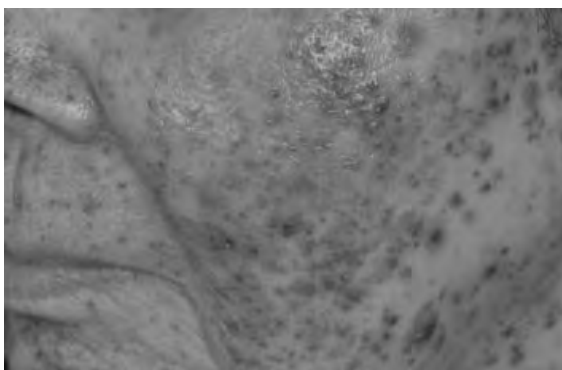


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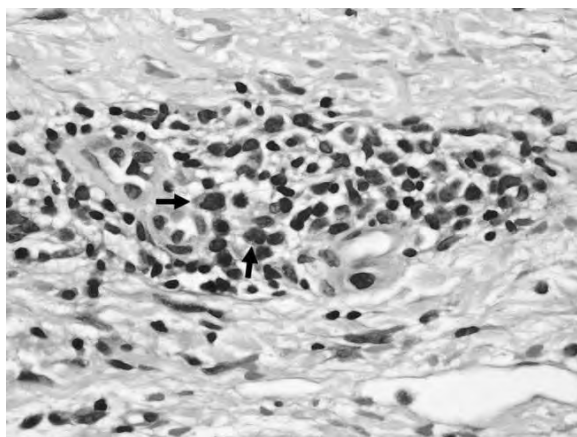


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Address for Correspondence: Dr Yee Kiat Heng, Department of Dermatology,
National Skin Centre, 1 Mandalay Road, Singapore 308205.
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