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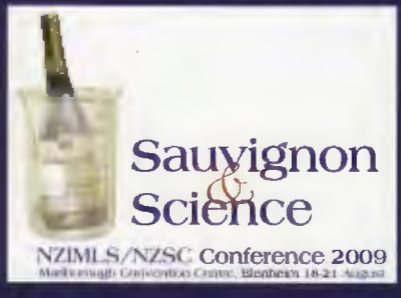

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Massey University NZIMLS Scholarship 2008

Hanan Aly is the winner of the New Zealand Institute of Medical Laboratory Science sponsored scholarship for the top third year student in the Massey University BMLSc program. The prize of $2,000 will be used by Hanan to support her studies during the final year of the program during which she will study haematology at MedLab Central and medical microbiology at Southern Community Laboratories in Dunedin.

My name is Hanan Aly which in Arabic means "Mercy, Compassion". I was born on September 1st, 1988 in Kuwait. I moved with my family to Palmerston North when I was 4 years old, at the time unknowing I would spend the next 17 years of my life here. I attended West End primary school and Intermediate Normal School before moving on to secondary education at Palmerston North Girls' High School. While there I took mostly science subjects as I wanted to pursue a career somewhere in the health science field.

Born the youngest of six siblings, each of whom had studied at university, I naturally followed in their footsteps and commenced my studies at Massey University. At university I took an interest in the biological sciences – liking everything from microbiology to physiology. It was obvious that I had made the right choice for myself with the BMLSc course, as it covered a lot of topics that I enjoyed. I look forward to graduating soon. Outside interests include reading, spending time with my family, photography, cooking and walking. My plans for the immediate future are to keep working and to improve my skills in the laboratory.

In this issue

Jones and Legge determined bone activity in physically active and sedentary spinal cord injured males by using biochemical markers of formation and resorption, namely bone alkaline phosphatase and deoxypyridinoline respectively. They found that the sedentary group had significantly higher bone alkaline phosphatise levels which was inversely related to activity levels and positively related to time post-injury.

Omoregie and Eghafona determined the prevalence of asymptomatic urinary tract infection (UTI) and anaemia among HIV and non-HIV subjects as well as the effect of asymptomatic UTI on the prevalence of anaemia. They found that HIV patients on highly active antiretroviral therapy (HAART) had a significantly higher prevalence of asymptomatic UTI. Anaemia prevalence was higher among HAART-naive HIV patients.

In this issue Sujata Hemmady presents a case study of adrenal carcinoma. The primary diagnosis of the patient was made after a complete endocrinology workup in addition to the analysis of catecholamine excretion and localized tumour imaging to rule out the possibility of phaeochromocytoma.

Another journal questionnaire appears in this issue. It remains a popular avenue to gain CPD points with a record 658 members completing the April 2009 journal questionnaire. A number of problems have been reported regarding the submission of the questionnaire in the past. While these are reducing, one major issue still remains. The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try resubmitting from a computer or system using Internet Explorer. Also, Michael Legge presents four laboratory medicine puzzles with questions for members to solve and the Haematology SIG has its usual questionnaire based on a journal article.

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Biochemical markers of bone activity in active and sedentary spinal cord injured men

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Abstract
Aim: To determine bone activity in spinal cord injured males by using biochemical markers of formation, bone alkaline phosphatase (BAP) and resorption, deoxypyridinoline (Dpd).
Methods: The markers were detected using enzyme-linked immunosorbent assays (ELISA) in eleven physically active and four sedentary spinal cord injured men.
Results: The sedentary group demonstrated significantly higher BAP when compared with the active group of SCI, (mean + SD; 28.0 + 6.4 U/l vs 14.0 + 3.6 U/l, respectively; $p<0.01$). A positive correlation was identified for time post-injury (TPI) and BAP activity for all subjects ($r=0.60$; $p<0.05$, $n=15$), while a negative correlation was found between activity levels and BAP activity ($r=-0.56$, $p<0.05$, $n=15$). Additionally, a positive association between bone resorption (Dpd) and time post injury in the active group was evidenced by a moderate correlation ($r=0.57$), however this failed to reach significance ($p=0.065$).
Conclusions: Further investigation is required to establish the effect of physical activity on bone metabolism in SCI and the usefulness of biochemical markers of bone activity in this population.

Key words: bone markers, biochemistry, bone alkaline phosphatase, deoxypyridinoline, spinal cord injury

N Z J Med Lab Sci 2009; 63: 40-43

Introduction

Disruption to the flow of information from the central nervous system and a marked lack of gravitationally influenced mechanical stresses create a unique form of osteoporosis in spinal cord injured (SCI) persons (1-4), characterised by a specific pattern of bone loss below the level of the lesion (5-7). Typically up to 33 per cent of bone mass is lost within the first six moths of injury, stabilizing to approximately 66 per cent of the original bone mass by 12 to 16 months post-injury, which is considered to be close to the fracture threshold of bone (7).

In the able-bodied population, bone mineral density is maintained by physical activity and the combination of force exerted via the long bones and active muscle tensions (8-10). The immobility as a result of spinal cord injury means that any physical activity has to be performed in the seated position thereby removing the gravitational influence on physical activity. Although bone mineral density has been measured in SCI individuals using dual energy X-ray absorptiometry (DXA), this technique does not provide an indicator of bone activity at the cellular level. Previous research using biomarkers of bone activity in SCI have focused on changes in bone mineral density from initial injury to rehabilitation (4, 11,12), but have not investigated the influence of exercise on bone mineral turnover.

In this research we have investigated the use of two bone biomarkers, one for bone reabsorption (urinary deoxypyridinoline, Dpd) and the other for bone formation (bone specific alkaline phosphatase, BAP) in sedentary and physically active SCI men, to establish the rate of bone turnover. This will provide an insight into the effects of active versus sedentary lifestyles in SCI individuals and may have implications in the future treatment of these injuries.

Materials and methods

Participants

Fifteen SCI males participated in this study. Fourteen were chronic, post-traumatic SCI and one classified as sensory and motor incomplete as a result of spinal artery thrombosis (Table 1). They were assigned to one of two groups, active (n=11) or sedentary (n=4) according to their self-reported weekly activity.

The active group reported a mean activity of 12.2 hours/week (± 6.0) whereas the sedentary group did not report any significant physical activity. Physical activity levels were assessed using a questionnaire. Ethical approval for this research was obtained from the Otago Southern Regional Health Authority Ethics Committee.

Bone biomarkers

For the bone reabsorption marker, the Dpd concentration was determined using a Pyrolinks™ (Metra Biosystems, Mountain View, California) immuno-assay kit from the first void morning urine sample, according to the manufacturers instructions. All urine samples were measured in duplicate and the results expressed as nmol Dpd/mmol creatinine (13).

To determine the rate of bone formation, the bone alkaline phosphatase (BAP) was measured in serum using a competitive immunoabsorbent assay specific for BAP (Metra Biosystems Alkphase-BTM , Mountain View, California). Results were expressed as U/l (14,15).

Statistical analysis

All statistical tests were undertaken using the SPSS statistical package (SPSS Inc, v14.0, Chicago, IL). Shapiro-Wilk testing of normality of data distribution was undertaken. Data for TPI, BAP and Dpd were found to violate the assumption of normality of distribution, therefore non-parametric tests were performed.

Mann-Whitney U tests were used to detect significance between the active and the sedentary groups for TPI, activity levels, bone reabsorption and bone formation. Spearman rank order correlation was used to detect relationships between tome post injury, activity levels, bone reabsorption and bone formation in combined data (n=15) and for the active group (n=11); numbers were to small to perform correlational analysis in the sedentary group (n=4). Significance was accepted at $p<0.05$.

Results

Demographic details for all participants is presented in Table 1.

Table 1. Characteristics of male spinal cord injured subjects				
Subject	Age (Years)	TPI (Years)	Level of lesion	Exercise (Hrs/wk)
1	26	4	C6/7 (I)	4
2	31	9	C5/6 (I)	11
3	35	19	C5/6 (C)	12
4	32	18	C5/6 (C)	22.5
5	28	12.5	C5/6 (C)	16
6	19	4	C6/7 (C)	10
7	23	4	C5/6 (I)	12
8	40	29	T5 (I)	5
9	31	1	L1-3 (C)	12
10	23	1.3	T12/L1 (I)	8
11	33	1.8	L1 (I)	22
12	58	36	T6 (C)	0
13	35	5	T12 (I)	0
14	51	32	C8/T1 (I)	0
15	54	35	T12/L1 (C)	0

(I) = Incomplete; (C) = Complete; TPI = time post injury

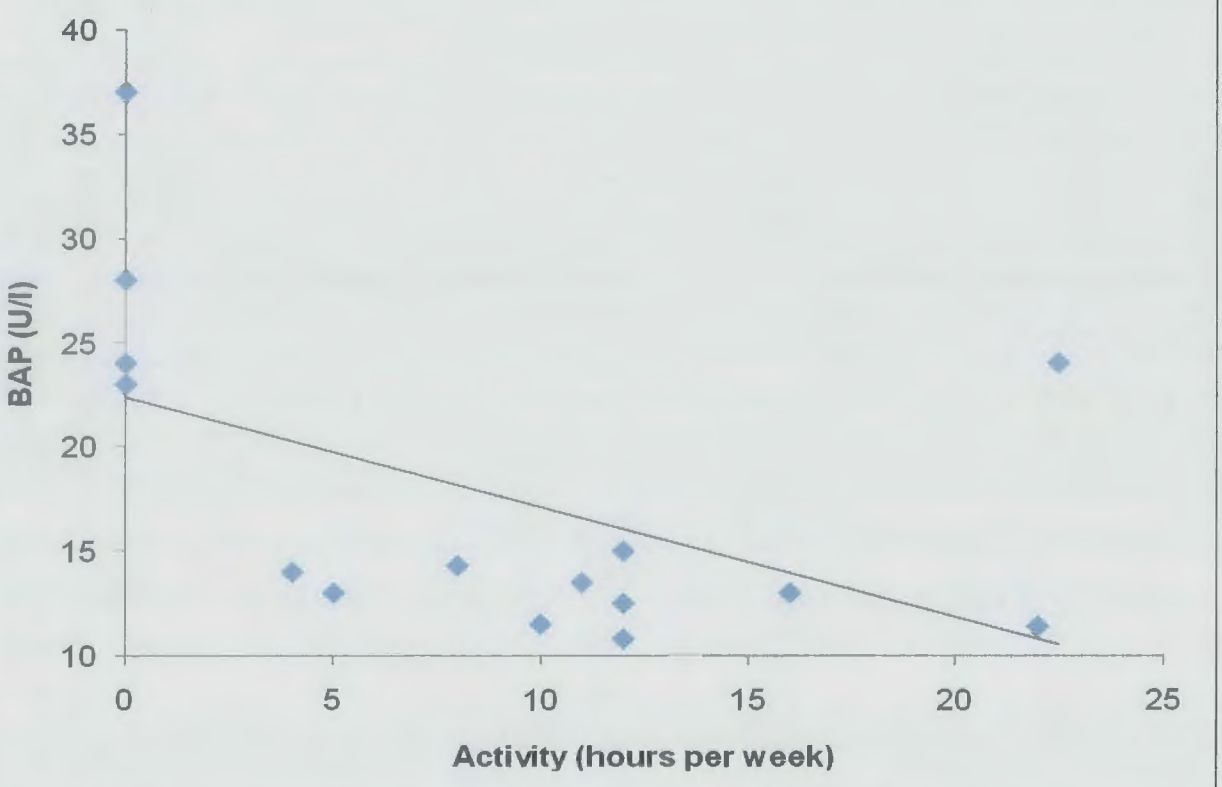

Figure 1. Scatter plot of the relationship (r=-056, p<0.05) between activity levels and the bone formation marker (bone alkaline phosphatase, BAP)

Data are presented as mean + standard deviation (SD) for clarity, with significance values reported from the Mann-Whitney U test or Spearman correlational analysis. The self reported activity levels between the active and the sedentary groups for time spent exercising per week was significant (12.2 ± 5.6 vs 0 hours per week, $p<0.01$), as expected. Sedentary SCI had sustained their injury for a longer duration than the active SCI group (27.0 ± 14.8 vs 9.6 ± 9.0 years, $p<0.05$), and were older (50 + 10 vs 29 + 6 years, $p<0.01$), respectively

Bone re-absorption
No significant differences were found for the bone reabsorption marker between the active and the sedentary SCI groups ($p>0.05$). However, the mean Dpd concentration in the active group (16.8 nmol/mmol creatinine ± 10.3) was higher than that in the sedentary group (11.8 nmol/mmol creatinine ± 5.4). In the active group, a moderate positive correlation that approached significance was found between TPI and bone resorption ($r=0.57$; $p=0.065$).

Bone formation
BAP activity in the sedentary SCI men was significantly higher than those of the active group (sedentary: 28.0 ± 6.4 U/l; active: 14.0 ± 3.6 U/l, $p<0.01$). When data were combined ($n=15$), significant correlations were found between bone formation and activity hours ($r=-.51$, $p<0.05$; Figure 1) and TPI ($r=0.60$, $p<0.05$).

Discussion
Early bone loss associated with spinal cord injury is from the entire skeleton with osteopenia prevalent primarily below the level of the lesion. It would be anticipated therefore that the bone biomarkers, such as Dpd and BAP would give an indication of the net changes of bone cellular activity (16).

Sedentary SCI subjects in this study had higher bone marker formation (BAP) rates than the active SCI subjects, a result which contrasts with studies in able-bodied athletes, in whom exercise has had a positive influence on bone density formation (9,10). While the sedentary SCI group in this study had higher bone formation rates, they had also sustained their injury for a longer time and were older. Previous research has shown that bone formation decreases progressively to a low at 60 days post-injury and reaches a new steady state at approximately 16 to 24 months post-injury (1,11,17), which is maintained to at least 10 years post-injury (7). Bed rest per se is not reported to influence the BAP marker, although these negative findings may have been influenced by the presence of other forms of alkaline phosphatise (18,19).

It is difficult therefore, to ascertain what influence (if any) exercise is having on these subjects. It may well be that duration of the injury plays a greater role in maintaining normal bone formation rates and that the active group may approach these rates later on. This is supported; in part when the sedentary and the active groups are combined, as a significant correlation ($r=0.60$, $p<0.05$) was found suggesting bone formation rates increase with time post injury. Conversely, combined group data demonstrated that as activity hours increased, bone formation rates decreased which may be due to the total amount and intensity of exercise being undertaken by the active group.

Previously, it has been reported that bone formation decreased in able-bodied men and women for up to two days following a single session of endurance exercise, but returned to normal activity thereafter (20). In the SCI subjects in the present study, repetitive bouts of exercise may have had a compounding effect on bone remodelling, preventing a return to baseline formation levels. Sedentary SCI in this study had bone formation rates within the population reference range.

Whilst not statistically significant ($p=0.85$), the bone reabsorption marker (Dpd) had a higher concentration in active SCI compared with the sedentary group (mean – 16.2 + 10.3 vs 11.8 + 5.4 nmol Dpd/mmol creatinine, respectively). Both groups had values outside the able-bodied males reference range (2.5 to 5.5nmol Dpd/mmol creatinine). Whilst a transient increase in bone reabsorption biomarkers has been identified in able-bodied athletes following intensive exercise (20), both the SCI groups in the present study demonstrate a high level of continuous bone reabsorption.

Although not statistically significant, a trend towards increasing bone resorption with the duration of injury was observed for the active group, but no such association was evident with total activity hours. Previous research has utilised data modelled on mathematical regressions lines deduced from DEXA measurements to predict bone mineral activity. This method, linked to the use of electrically stimulated exercise, has been shown to improve muscle function but does not however, improve the prediction of decreasing bone mineral density in the SCI (21-23), leading to an underestimation in bone reabsorption.

A significant limitation in this study was the low number of sedentary SCI, which did not allow a more rigorous statistical investigation of the data. Despite this, the results presented do provide evidence that remodelling dynamics differ in SCI individuals and vary dependant on physical activity and duration of injury. Further work to validate these results needs to be undertaken, extending these preliminary findings to a larger athletic SCI population to establish how duration and intensity of exercise, and age and duration of injury may affect bone remodelling. We also would caution the use of single measurements of biomarkers of bone remodelling in the SCI population who clearly demonstrate different bone dynamics than seen in the able-bodied population.

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References
1. Chantraine A, Nusgens B, Lapiere CM. Bone remodeling during the development of osteoporosis in paraplegia. *Calcif Tissue Int* 1986; 38: 323-7.
2. Hill EL, Martin RB, Gunther E, Morey-Holton E, Holets VR. Changes in bone in a model of spinal cord injury. *J Orthop Res* 1993; 11: 537-47.
3. Minaire P, Meunier P, Edouard C, et al. Quantitative histological data on disuse osteoporosis. Comparison with biological data. *Calcif Tissue Res* 1974; 17: 57-73.
4. Uebelhart D, Demiaux-Domenech B, Roth M, et al. Prospective evolution of biochemical markers of bone metabolism and whole-body composition in spinal cord injury. *J Bone Min Res* 1995; 10: S181.
5. Biering-Sorensen F, Bohr H, Schaadt O. Bone mineral content of the lumbar spine and lower extremities years after spinal cord lesion. *Paraplegia* 1988; 26: 293-301.
6. Finsen V, Indredavik B, Fougner KJ. Bone mineral and hormone status in paraplegics. *Paraplegia* 1992; 30: 343-7.
7. Garland DE, Stewart CA, Adkins RH, et al. Osteoporosis after spinal cord injury. *J Orthop Res* 1992; 10: 371-8.
8. Block JE, Friedlander AL, Brooks GA, et al. Determinants of bone density among athletes engaged in weight-bearing and non weight-bearing activity. *J Appl Physiol* 1989; 67: 1100-5.
9. Hamdy RC, Anderson JS, Whalen KE, Harvill LM. Regional differences in bone density of young men involved in different exercises. *Med Sci Sports Exerc* 1994; 26: 884-8.
10. Menkes A, Mazel S, Redmond RA, Pratley RE, Hurley BF. Strength training increases regional bone mineral density and remodeling in middle-aged and older men. *J Appl Physiol* 1993; 74: 2478-84.

11. Uebelhart D, Demiaux-Domenech B, Roth M, Chantraine A. Bone metabolism in spinal cord injured individuals and in others who have prolonged immobilisation. A review. *Paraplegia* 1995; 33: 669-73.
12. Uebelhart D, Hartmann D, Vuagnat H, et al. Early modifications of biochemical markers of bone metabolism in spinal cord injury patients. A preliminary study. Scand *J Rehabil Med* 1994; 26: 197-202.
13. Kamel S, Brazier M, Neri V, et al. Multiple molecular forms of pyridinolines crosslinks excreted in human urine evaluated by chromatographic and immunoassay methods. *J Bone Min Res* 1995; 10: 1385-92.
14. Gomez Jr B, Ardakani S, Ju J, et al. Monoclonal antibody assay for measuring bone-specific alkaline phosphatase activity in serum. *Clin Chem* 1995; 41: 1560-6.
15. Gomez Jr B, Haugen S, Ardakani S, et al. Measurement of bone-specific alkaline phosphatase activity in serum using a monoclonal antibody. *J Bone Min Res* 1994; 9: S348.
16. Maimoun L, Couret I, Micallef J-P, et al. Use of biochemical markers with dual-energy x-ray absorptiometry for early determination of bone loss in persons with spinal cord injury. *Metabolism* 2002; 51: 958-63.
17. Biering-Sorensen F, Bohr HH, Schaadt OP. Longitudinal study of bone mineral content in the lumbar spine, the forearm and the lower extremities after spinal cord injury. *Eur J Clin Invest* 1990; 20: 330-5.
18. Leuken SA, Arnaud SB, Taylor AK, Baylink DJ. Changes in markers of bone formation and resorption in a bedrest model of weightlessness. *J Bone Min Res* 1993; 8: 1433-8.
19. van der Wiel HE, Lips P, Nauta J, Netelenbos JC, Hazenburg GJ. Biochemical parameters of bone turnover during ten days of bed rest and subsequent mobilisation. *Bone Min* 1991; 13: 123-9.
20 Brahm H, Piehl-Aulin K, Ljunghall S. Biochemical markers of bone metabolism during distance running in healthy, regularly exercising men and women. Scand *J Med Exerc Sport* 1996; 6: 26-30.
21. Hangartner TN, Rodgers MM, Glaser RM, Barre PS. Tibial bone density loss in spinal cord injured patients: Effects of FES exercise. *J Rehabil Res Dev* 1994; 31: 50-61.
22. Leeds EM, Klose KJ, Ganz W, Serafini A, Green BA. Bone mineral density after bicycle ergometry training. *Arch Phys Med Rehabil* 1990; 71: 207-9.
23. Rodgers MM, Glaser RM, Figoni SF, et al. Musculoskeletal responses of spinal cord injured individuals to functional neuromuscular stimulation-induced knee extension exercise training. *J Rehabil Res Dev* 1991; 28: 19-26.

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Effect of urinary tract infection on the prevalence of anaemia among HIV patients in Benin City, Nigeria

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Abstract
Objectives: To determine the prevalence of asymptomatic urinary tract infection (UTI) and anaemia among human immunodeficiency virus (HIV) and non-HIV subjects as well as the effect of asymptomatic UTI on the prevalence of anaemia.
Methods: Clean-catch midstream urine and venous blood specimens were collected from 421 subjects consisting of 216 HIV patients on highly active antiretroviral therapy (HAART), 101 HAART naive HIV patients and 104 apparently healthy non-HIV subjects. Urine specimens were processed to diagnose asymptomatic UTI, while the blood specimens were processed for haemoglobin (Hb) estimation. Anaemia was defined as Hb concentration <130g/L for males and <120g/L for females.
Results: HIV patients on HAART had significantly higher (p=0.046) prevalence of asymptomatic UTI compared with non-HIV subjects (27.8% vs 17.3%). HAART naive HIV patients had significantly higher prevalence of anaemia (79.2%) compared with those on HAART (44.4%) and non-HIV subjects (33.6%) (p<0.0001). The prevalence of anaemia was not significantly affected by asymptomatic UTI among the various groups of the study population (p>0.05).
Conclusions: An overall prevalence of 24.9% and 50.1% of asymptomatic UTI and anaemia respectively was recorded. Prevalence of asymptomatic UTI was higher among HIV patients on HAART while prevalence for anaemia was higher among HAART naive HIV patients. Asymptomatic UTI had no effect on the prevalence of anaemia in both HIV and non-HIV subjects.

Key words: HIV, urinary tract infection, anaemia, Nigeria

N Z J Med Lab Sci 2009: 63: 44-46

Introduction
Anaemia is the most commonly encountered haematological abnormality in human immunodeficiency virus (HIV) patients, occurs with increasing frequency and is a significant predictor of progression towards acquired immunodeficiency syndrome (AIDS) or death, with more than 70% of patients developing anaemia and requiring transfusion (1,2). Highly active antiretroviral therapy (HAART) entails treatment with a combination of two nucleoside reverse transcriptase inhibitors and a potent protease or non-nucleoside reverse transcriptase inhibitors, and is generally the gold standard for the management of HIV patients (2). HAART has been reported to increase the haemoglobin concentration and to reduce the prevalence of anaemia (2,3). However, among HIV patients on HAART, anaemia is still being reported (4,5).

The cause of anaemia in HIV patients is multi-factorial and includes infections, neoplasms, dietary deficiencies, blood loss, and medications (6). Urinary tract infection (UTI) is one of the infections observed among HIV patients, although reports on the impact of HIV on the prevalence of UTI are conflicting (7,8). Renal diseases have also been reported among HIV/AIDS patients (9). It is expected that renal damage in HIV patients will affect erythropoietin production and ultimately result in anaemia. To our knowledge, there are no published studies on the effect of UTI on the prevalence of anaemia among HIV patients. Hence this study set out to determine the prevalence of UTI and anaemia among HIV and non-HIV subjects as well as the effect of UTI on the prevalence of anaemia in both populations.

Methods
Study population
This study was carried out at the University of Benin Teaching Hospital, Benin City, Nigeria. A total 421 subjects were studied consisting of 317 HIV patients and 104 apparently healthy HIV sero-negative individuals. The HIV patients consisted of 216 patients on HAART for 3 – 6months and 101 HAART naive patients. The HIV patients were asymptomatic and all subjects had no sign or symptom of UTI and anaemia. Exclusion criteria include antibiotic usage within one week and large fluid in-take (in previous hour) before clinic attendance. The HAART regimen for HIV patients on HAART consisted of zidovudine, stavudine and nevirapine. Verbal informed consent was obtained from all subject before specimen collection. The study was approved by the Ethical Committee of the University of Benin Teaching Hospital.

Specimen collection and processing
Clean-catch mid-stream urine and 5 mL of venous blood was collected from each subject. Urine specimens were collected into a sterile screw-capped universal container containing a few crystals of boric acid as preservative. The blood specimens were collected in ethylene diamine tetra-acetic acid (EDTA) bottles.

A loopful (0.001mL) of well mixed un-centrifuged urine was streaked onto the surface of blood agar and cystine lactose electrolyte deficient (CLED) medium (M6: Plasmatec Laboratories, United Kingdom). The plates were incubated aerobically at 370C for 24 hours and counts were expressed in colony forming units (CFU) per mL. A count of ≥105CFU/mL was considered to indicate asymptomatic UTI. Ten mL of each well-mixed urine sample was centrifuged at 2000g for 5mins. The supernatant was discarded and a drop of the deposit was examined microscopically at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals, yeast-like cells and Trichomonas vaginalis. Pus cells ≥5 per higher power field was considered to indicate infection.

Blood samples were analysed for haemoglobin with a Sysmex KX – 21 haematology analyzer (Sysmex Corporation, Kobe, Japan).

Table 1. Prevalence of asymptomatic urinary tract infection and anaemia			
	Non-HIV (n = 104)	HAART naive (n = 101)	On HAART (n = 216)
UTI	18 (17.3%)	27 (26.7%)	60 (27.8%)*
Anaemia	35 (33.7%)	80 (79.2%)+,#	96 (44.4%)

Figures in parenthesis are percentages
* On HAART vs non—HIV: p = 0.046
+ HAART naÔve vs Non-HIV: p<0.0001
# HAART naive vs On HAART: p < 0.0001

Table 2. Effect of urinary tract infection on prevalence of anaemia.			
Subject		Number Tested	Number with anaemia (%)
Non-HIV	with UT	18	9 (50.0%)
	without UTI	86	26 (30.2%)
HAART naive	with UT	27	24 (88.9%)+,#
	without UTI	74	56 (75.7%)
On HAART	with UT	60	26 (43.3%)
	without UTI	156	70 (44.9%)

+ HAART naive vs non-HIV: P = 0.0061
# HAART naive vs on HAART: p < 0.0001

Anaemia was defined according to WHO criteria (10). For males this was a haemoglobin concentration of less than 130g/L, for females less than 120g/L.

Statistical Analysis
Data was analysed using Chi (X2) square test or Fisher's exact test using the statistical software INSTAT. Statistical significance was set at the p 0.05 level.

Results
Only HIV patients on HAART had a significantly higher prevalence of UTI compared with their non-HIV counterparts (27.8% vs 17.3% respectively; p=0.046). As shown in Table 1 the prevalence of anaemia was significantly higher among HAART naive HIV patients compared with those on HAART and non-HIV subjects.

The prevalence of anaemia in all study subjects was not significantly affected by asymptomatic UTI. However, as shown in Table 2, the prevalence of anaemia among HAART naive HIV patients with UTI was significantly higher than those of non-HIV subjects and HIV patients on HAART.

Discussion
Against the background of infection (of which UTI is one) being one of the causes of anaemia among HIV patients (6), this study focused on determining the prevalence of asymptomatic UTI and anaemia among HIV and non-HIV subjects as well as the effect of UTI on the prevalence of anaemia in the study population.

In our study, only HIV patients on HAART had significantly higher prevalence of asymptomatic UTI compared with non-HIV subject. It is known that the antibacterial property of human urine lies in its low pH, high urine urea concentration and osmolality (11). It has been reported that some anti-retrovirals used in the HAART regimen results in crystalluria, nephrolithiasis, decreased glomerular filtration rate and decreased urine osmolality (12). This may affect the antibacterial properly of urine and may explain our results.

The prevalence of asymptomatic UTI among HAART naive HIV patients was higher than that for non-HIV subjects, though failing to reach statistical significance. This finding is in agreement with earlier reports (7). Reports that showed higher prevalence of UTI either studied AIDS patients, or HIV patients with symptoms of UTI (8,13).

The prevalence of anaemia among HAART naive HIV patients was significantly higher than non-HIV subjects and HIV patients on HAART. The finding that apparently healthy individuals (even those without UTI) had anaemia agrees with an earlier report and a worsening economy was suggested as a possible reason (14). Bone marrow suppression, especially the erythroid lines, have been reported among other mechanisms as the cause of anaemia among HIV patients (2,6). With HIV patients on HAART, inclusion of zidovudine in the HAART regimen and production of antibodies against HAART agents have also been reported as possible mechanisms (4,6,14). Zidovudine is among the HAART agents used by HIV patients in this study.

The presence of asymptomatic UTI did not affect the prevalence of anaemia in both HIV and non-HIV subjects. A broad spectrum of renal diseases had been reported in patients with HIV/AIDS (9). It is expected that renal damage in HIV patients will affect erythropoietin production and ultimately result in anaemia. This was not observed in this study and may be due to the fact that our patients were asymptomatic and as such may not have developed renal complications. An assessment of renal function of

such patients is needed to confirm this. The higher prevalence of anaemia among HAART naive HIV patients with UTI in comparison to other populations in this study is therefore not due to UTI, but to HIV infection itself. HAART naive HIV patients should be placed on HAART, preferably a regimen without zidovudine, to reduce the prevalence of anaemia and improve the quality of life of HIV patients (2).

In conclusion, an overall prevalence of asymptomatic UTI and anaemia of 24.9% and 50.1% respectively was observed in this study. The prevalence of asymptomatic UTI was higher among HIV patients on HAART, while the prevalence of anaemia was higher among their HAART naive counterparts. Asymptomatic UTI had no effect on the prevalence of anaemia.

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References

1. Volberding P. The impact of anemia on quality of life in human immunodeficiency virus-infected patients. *J Infect Dis* 2002; 185 (Suppl 2): S110 -4.
2. Odunukwe N, Idigbe O, Kanki P, Adewole T, Onwujekwe D, Audu R, et al. Haematological and biochemical response to treatment of HIV-1 infection with a combination of nevirapine + stavudine + lamivudine in Lagos Nigeria. *Turk J Haematol* 2005; 22: 125 – 31.
3. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systemic review of the literature *Am J Med* 2004; 116 (Suppl 7A): 27S – 43S.
4. Curkendall SM, Richardson JT, Emons MF, Fisher AE, Everhard F. Incidence of anaemia among HIV-infected patients treated with highly active antiretroviral therapy. *HIV Med* 2007; 8: 483 – 90.
5. Mildvan D. Implications of anemia in human immunodeficiency virus, cancer, and hepatitis C virus. *Clin Infect Dis* 2003; 37 (Suppl. 4): S293 – 6.
6. Moyle G. Anaemia in persons with HIV infection: prognostic marker and contributor to morbidity. *AIDS Rev* 2002; 4: 13 – 20.
7. Park JC, Buono D, Smith DK, Peipert JF, Sobel J, Rompalo A., et al. Urinary tract infections in women with or at risk for human immunodeficiency virus infection. *Am J Obstet Gynecol* 2002; 187: 581 – 8.
8. Pinho AM, Lopes GS, Ramos-Filho CF, Santos Oda R, De Oliviera MP, Halpern M, et al. Urinary tract infection in men with AIDS. *Genitourin Med* 1994; 70: 30-4.
9. Janakiraman H, Abraham G, Mathew M, Kuruvilla S, Panikar V, Solomon S, et al. Correlation of CD4 counts with renal disease in HIV positive patients. *Saudi J Kidney Dis Transpl* 2008; 19: 603 – 7.
10. Izaks GJ, Westendorp RG., Knook DL. The definition of anemia in older persons. *JAMA* 1999; 281: 1714 – 7.
11. Kaye D. Antibacterial activity of human urine. *J Clin Invest* 1968; 47: 2374 – 90.
12. Eira M, Araujo M, Seguro AC. Urinary NO3 excretion and renal failure in indinavir-treated patients. *Braz J Med Biol Res* 2006; 39: 1065 – 70.
13. Evans JK, McOwan A, Hillman RJ, Forster GE. Incidence of symptomatic urinary tract infections in HIV seropositive patients and the use of cotrimoxazole as prophylaxis against Pneumocystis carinii pneumonia. *Genitourin Med* 1995; 71: 120 – 2.
14. Olayemi E, Halim NK, Anaemia in apparently healthy adult Nigerians. *J Coll Med* 2005; 10: 31 – 33.
15. Omoregie R, Egbeobauwaye A, Ogefere H, Omokaro EU, Ekeh CC. Prevalence of antibodies to HAART agents among HIV patients in Benin City, Nigeria. *Afr J Biomed Res* 2008; 11: 33 – 7.

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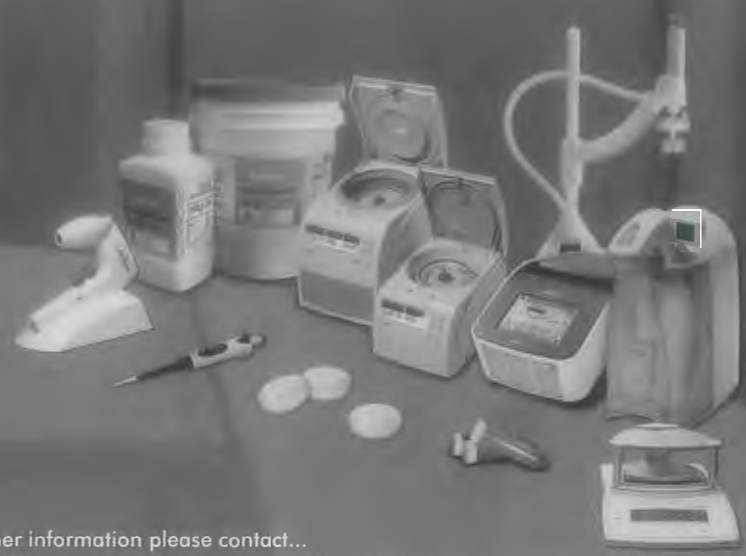

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Adrenal carcinoma: a case study

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Abstract

Adrenocortical masses are common, may be clinically asymptomatic and are often found incidentally as a result of unrelated imaging investigations. The evaluation of such tumours focuses on identifying the carcinoma early with the intent of complete surgical removal if required and subsequent follow up. The purpose of this case study was to evaluate the function and role of various hormones by interpreting laboratory results and to correlate the manifestation of symptoms that are usually associated with adrenal carcinoma.

Key words: Adrenal carcinoma, cortisol, catecholamine, adrenal incidentaloma, adrenocorticotropin hormone, dehydroepiandrosterone, androstenedione, testosterone, phaeochromocytoma

N Z J Med Lab Sci 2009; 63 (2): 48-50

Introduction

Adrenal carcinoma is uncommon and accounts for approximately 0.6-1.67 cases per million persons per year with the exception of the inordinately high frequency (up to 10-fold higher) of cases among children in southern Brazil (1). While adrenal carcinoma accounts for only approximately 5-10% of cases of Cushing syndrome; approximately 40% of patients with both Cushing syndrome and an adrenal mass have a malignant tumour (2, 3). Adrenocortical carcinoma usually presents with dysfunctional uterine bleeding in women with increased amounts of androstenedione and estrogens that results in Cushing's syndrome, suggesting inefficient conversion of cortisol (4). Cortisol-producing adrenal adenoma is indicated by elevation in baseline urine free cortisol (UFC) and a corresponding reduction in plasma androgen secretion in the cortisol-induced suppression of adrenocorticotropin hormone (ACTH) and subsequent androgen producing zona reticularis of the adrenal gland. Whereas, adrenal carcinoma is indicated by a palpable abdomen with markedly elevated values of plasma dehydroepiandrosterone (DHEAS), urine free cortisol and is generally resistant to dexamethasone suppression. Elevated secretion of DHEAS often leads to virilization in the female (4).

Case study

Patient N, a 48-year old female first presented to the Emergency Department, Auckland Hospital in October 2007 with a pre-syncopal episode associated with palpitations and shortness of breath. Her symptoms had commenced two months prior to her first presentation and she had been started on propranolol. She was cold, clammy, sweaty and tachycardic, but her blood pressure was normal. She presented again, almost two months later with severe abdominal pain and her ECG showed broad complex tachycardia with a heart rate of 210/min. The patient continued to have broad complex tachycardia with pre-syncope and hypertension (170/110 mmHg) despite treatment. She had a palpable abdominal mass and was subsequently admitted for a series of screening laboratory tests that included a complete endocrinology workup. A CT scan of the patient's adrenal gland confirmed the presence of a 5 cm mass arising either from the adrenal or the kidney.

Methods

The endocrinology screening tests were analysed on a Siemens Immulite 2000 Automated Immunoassay Analyzer.

Urinary catecholamine quantitation was undertaken by HPLC on an acidified 24 hour urine collection. 3, 4-Dihydroxybenzlamine (DHBA) and catecholamine mixed stock solution (Noradrenalin 150 µmol/L, Adrenaline 150 µmol/L and Dopamine 750 µmol/L) was used to prepare an electro active internal standard. Prior to injection the urine was pre-treated on a Biorex 70 column (Bio-Rad) and the eluate injected into the HPLC reverse phase column 150mm long with an internal diameter of 3.2 mm (Phenomenex Prodigy, USA), using octylsulphonate as an ion pair agent for separation on a Waters 2690 Alliance separation module.

Laboratory results

After preliminary investigations, plasma ACTH and free 24 hour urinary cortisol for Cushing's disease was initiated although the patient displayed no clinical features or symptoms of the disease apart from elevated blood pressure. In addition, plasma aldosterone was determined for hyperaldosteronism. Androstenedione, DHEAS, testosterone (including free) and sex hormone binding globulin (SHBG) were assayed to exclude virilization syndrome. The laboratory investigations revealed an elevated 24hour urine free cortisol level and normal catecholamine levels (Tables 1 and 2). Plasma ACTH was low and DHEA, androstenedione and testosterone were all elevated.

Histology

Although the initial laboratory investigations indicated the possibility of an adrenocortical tumour, this was difficult to confirm as histological analysis of either an adenoma or carcinoma is often unreliable and FNA or core biopsies are generally not recommended in the diagnostic workup due to a likelihood of the tumour seeding into the retroperitoneum (5). However, it was decided that the next step for evaluation was to perform a CT guided core biopsy to confirm the diagnosis of an adrenal carcinoma which was subsequently performed on Patient N.

The histological examination of the core biopsy revealed a pronounced dystrophic calcification, which was negative for pancytokeratin and chromogranin but positive for synaptophysin. It was decided that the tumour did not fit with any type of renal carcinoma as there was no evidence of papillae formations generally associated with renal carcinoma or the diagnostic immunoprofile for a papillary carcinoma. Phaeochromocytoma was finally excluded due to the presence of a basophilic cytoplasm and the obvious absence of S100 positive sustentacular cells. The tumour should have been equally strongly positive for chromogranin and synaptophysin if it were phaeochromocytoma. It was concluded that the tumour represented an adrenal cortical carcinoma as it had the correct haematoxylin and eosin appearance for an adrenal cortical tumour.

Discussion

There have been several cases where catecholamine-secreting tumours have been under diagnosed due to a marked variation in the intermittent nature of the presenting symptoms. A similar case reported in 1990 indicated that adrenal cortical adenomas causing clinical features of phaeochromocytoma and elevated 24 hour urinary catecholamines have been reported rarely and that patients with hypertension and on long term medication of beta blockers may have an increased urinary norepinephrine excretion (6). Hence multiple 24 hours urine collections for catecholamines are recommended (7). To exclude the possibility of Patient N having

Table 1. Patient's laboratory results			
Assay	Result	Reference Interval	Remarks
Androstenedione (nmol/L)	1320	2.0 - 13.0	
DHEAS (µmol/L)	218	0.7 – 6.5	
ACTH (pmol/L)	<1.1	2.0 - 11.0	(at 0900 hrs)
Testosterone (nmol/L)	10.1	0.5 - 2.5	
SHBG (nmol/L)	8	18 - 114	(Adult non-pregnant)
Free testosterone (pmol/L)	341	0 - 50	(Calculated)
Aldosterone (pmol/L)	858 (Standing)	100 - 850	(Standing)
		50 - 450	(Recumbent)

Table 2 : Laboratory results of Patient N (24 hrs. urine)				
Assay	Result			Reference Interval
Urine free cortisol (nmol/L)	9432			100 - 380
	Urine catecholamines			
	I	II	III	
Adrenalin* (nmol/mmol creatinine)	0.46	0.58	0.51	0 - 9.5
Adrenalin (nmol/day)	9.0	11.0	12.0	0 - 100
Noradrenalin* (nmol/mmol creatinine)	38	29	33	0 - 69
Noradrenalin (nmol/day)	750	722	758	0 - 760
Dopamine* (nmol/mmol creatinine)	0.18	0.18	0.15	0 - 0.28
Dopamine (mol/day)	3.6	3.4	3.6	0 - 4.0

* expressed as a ratio to urinary creatinine

phaeochromocytoma, two more 24 hour urinary catecholamine tests were performed. The results showed catecholamine levels as normal; thereby excluding phaeochromocytoma (Table 2).

The exact etiopathogenesis of sporadic adrenal carcinoma is unclear. However, the role of tumour suppressor gene mutations is indicated by their association with Li-Fraumeni syndrome, which is characterized by inactivating germline mutations of the TP53 gene, gives some insight into the origins of these tumours (1, 3). This syndrome is associated with predisposition to adrenal carcinoma and other malignancies, including breast carcinoma, leukaemias, osteosarcomas and soft tissue sarcomas (3). It has also been proposed that adrenal hyperplasia predisposes patients to develop adrenal carcinoma, as a few cases of congenital adrenal hyperplasia are associated with functional adrenocortical adenomas but not carcinomas (6).

Among the putative pathogenic mechanisms that may function in concert are alterations in intracellular communication, paracrine and autocrine effects of various growth factors, cytokines elaborated by the tumour cells and promiscuous expression of various ligand receptors on the cell membranes of the cells that cause them to be in a state of perpetual hyper-stimulation. This is presumed to lead to clonal adrenal cellular hyperplasia, autonomous proliferation, tumour formation and hormone elaboration (2). Molecular studies of adrenocortical tumour cells show mutations of the tumour suppressor genes TP53, TP57 and increased production of insulin-like-growth factor 2 (2). Germ cell mutations of the P53 gene have also been demonstrated in more than 90% of children with adrenal carcinoma from southern Brazil, which has the highest prevalence of sporadic adrenal carcinoma in the world (1). Amplification of steroidogenic factor-1 expression has also been described in this population (3). Other studies demonstrate that some of these tumour cells express menin (MEN-1), the aberrant gene product in patients with multiple endocrine neoplasia type 1; in others, the hybrid gene is associated with glucocorticoid-responsive aldosteronism (3, 6). A few cases of adrenal carcinoma are associated with primary hyperaldosteronism, in which the adrenal tissue has portions showing hyperplasia (3, 6).

For treatment, mitotane remains the major chemotherapeutic option for the management of adrenal carcinoma because it is a relatively specific adrenocortical cytotoxin although its capacity to prolong clinical survival is uncertain. At best, only 20-25% of patients respond to mitotane (3). Advances in the treatment of adrenal carcinoma currently include an international phase III trial evaluating chemotherapy regimens, vascular growth inhibitors and small molecular pathways involved in tumour genesis.

Conclusions

The primary diagnosis of the patient was made after she underwent a complete endocrinology workup in addition to the analysis of catecholamine excretion and localized tumour imaging to rule out the possibility of phaeochromocytoma, a condition that results from a histological variant tumour of the same embryonic origin, typically mixtures of phaeochromocytoma spindle cell sarcomas and adrenocortical carcinomas (3). Although the means of identifying adrenal carcinoma is still controversial, virtually all authorities agree about removing all nonfunctional adrenal tumours larger than or equal to 6 cm because of significant cancer risk (5). The management strategy for adrenal masses larger than 3 cm and less than 6 cm is disputed, as was the case with this patient. Therefore, although the patient in this case study presented with no clinical features of Cushing's or virilization syndromes, it was imperative to distinguish potential adrenal carcinoma from an adrenal incidentaloma.

The laboratory investigations carried out for patient N led to a timely diagnosis that resulted in total resection as management modality although recurrent local metastatic disease is common in adrenal carcinoma.

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References

1. Sandrini R, Ribeiro RC, DeLacerda L. Childhood adrenocorticol tumors. *J Clin Endocrinol Metab* 1997; 82: 2027-31.
2. Ramzi C, Vinay K, Tucker C. Robbins. Pathologic Basis of Disease. WB Saunders, London, 1999.
3. Clark S, Orlo P, Komminoth P, Roth J, Schroder S et al. Endocrine Tumours. American Cancer Society Atlas of Oncology Series. Decker, New York, 2003.
4. Fauci A, Braunwald E, Kasper D, Hauser S (eds). Harrison's Principles of Internal Medicine. Mc Graw Hill, Philadelphia, 2008.
5. Cote R, Suster S, Weiss L, Weidner N, editors. Modern Surgical Pathology. WB Saunders, London, 2002.
6. Ross NS, Aron DC. Hormonal evaluation of the patient with an incidentally discovered adrenal mass. *N Eng J Med* 1990; 323: 1401-5.
7. Alsabeh R, Mazouijian G, Goates J, Medeiros LJ, Weiss LM. Adrenal cortical tumors clinically mimicking phaeochromocytoma. *Am J Clin Pathol* 1995; 104: 382-90.

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Phlebotomy and Specimen Services SIG

On 23rd May the Phlebotomy and Specimen Services SIG was held, in combination with the North Island Seminar, at the Waipuna Conference Centre, in Mt. Wellington, Auckland.

There was a wonderful turnout with over 180 people registered for the event. This annual event has become the highlight for many staff in our service who are seeking further development and knowledge. Not only were the North Island laboratories well represented, but there were many staff from the South Island laboratories as well. The program introduced a variety of topics including practical aspects, (how to use a venoscope), quality (how to comply with the NSZ/ISO15189 standard) and communication (how to communicate without the "wiggle room").

The program commenced with an introduction by Ross Hewitt, the Laboratory Manager at LabPlus, Auckland City Hospital. Ross was involved in a project with the NZIMLS in producing a video that would be available for schools as a way to introduce Medical Laboratory Science as a career option for school leavers. The video "starred" Kristen Kelly, the Quality Manager at LabPlus and a 7th form student who was contemplating what career pathway to embark on. The video was well presented, informative and provided a good overview of the type of work that is performed in medical laboratories throughout the country. It included routine analysis as well as some of the more specialised testing. There were plenty of visual effects that are aimed to capture the attention of the potential audience.

Peter Ford, the Clinical Sales Specialist for Becton-Dickinson (BD) in NZ, presented the topic "World-wide trends in specimen collection." His focus was on the newer products that can be used to help reduce needle-stick injuries and occupational health hazards. The evacuated urine system was an interesting concept that has practical potential and would be useful when urine specimens require "splitting" so the specimen can be shared between different laboratory sections/departments.

Elizabeth Le Page, a Phlebotomist working in the out-patient blood collection department at Hastings Hospital, discussed the use of a venoscope for trying to find those difficult and elusive veins. She purchased the Venoscope II over the internet from an Australian company. It resembles a stud-finder, no, not the male variety! The device is placed on the surface of the skin so that the light is directed down into the subcutaneous tissue. The ambient light is dimmed as much as possible so as to provide the contrast needed to see the vein. As the LED light illuminates the patient's subcutaneous tissue it highlights the veins which absorb the light rather that reflecting it. The device is powered by 3 AA alkaline batteries, and in Elizabeth's experience the batteries can give as much as 500 hrs of use. To get the knack of using the device it helps to practice on the good veins as well.

There is a lot of interest world-wide on Multi-resistant Organisms (MROs). This was the topic for Barbara Davidson's talk. Barbara is the Infection Control Nurse Specialist at Waitemata DHB and she works at North Shore Hospital. Barbara spoke specifically on the "Modes of Transmission" of ESBL (Extended Spectrum Beta-Lactamase), MRSA (Methicillin-Resistant Staphylococcus Aureus) and VRE (Vancomycin Resistant Enterococcus). Some, if not all, of these organisms are now endemic in many of New Zealand's hospitals. We were given an insight into the spread of these organisms through hands, cough secretions, droplets and surface contact. The susceptibility of patients in the hospital setting and the onerous job of screening were also discussed.

David Haines from Diagnostic MedLab in Auckland, provided us with an insight into the world of Quantiferon TB Gold. This test is a robust test and is used as a screening test for TB. It measures gamma interferon. In some facilities the Quantiferon Gold test has replaced the Tuberculin skin test (Mantoux test.), as the test of choice. Three blood tubes are collected as part of the test procedure; the gray-top vacuum blood collection tube is a blank SST tube, the blue-top tube is coated with mitogen to stimulate the T cells to divide, and the 3rd tube is a red-top tube containing TB antigen. The samples are handled at room temperature, but must be sent to the referral laboratory within 8 hrs of collection as they require a 16 hrs incubation period at 37ʃC. The specimens are then centrifuged and the supernatant stored for further processing. Samples are usually batched, then processed. The interpretation of the test is generally more clear-cut than the interpretation of the Mantoux test. The results are expressed as Positive (patient has TB infection), Negative (patient does not have TB) or Inconclusive (patient sample compromised? immuno-suppression). David provided some background on immune response mechanisms and discussed the infection process after exposure to TB; the dormant phase, active TB and the likely/expected outcomes following treatment or non-treatment. It was a very informative talk and leads us to the hidden world of lymphokines, macrophages and inflammatory mediators.

After such a stimulating morning, we took time-out for lunch. There was plenty of food with a good variety. The weather was fine and sunny, so many took their lunch outside to the patio area beside the Waipuna Lagoon. It was a good time to catch up with friends and colleagues.

Daphne Mason, the Laboratory Support Manager with Diagnostic MedLab Ltd in Auckland, provide us with some insights into Study and Exam Techniques. Preparation, as we all know is the key, and also being an active learner. Key pointers were to study little and often, summarise your notes, use acronyms, study with a "study buddy", tests yourself on old exam papers and make up questions for yourself. It is also important to remember that the QPT and QSST are national exams, and local practice and terminology may not be standard nationally. If you are unfamiliar with the exam venue, then an advance site visit is a good idea. Use the reading time wisely, make sure you understand the instructions, read each question carefully and calculate how much time to spend on each question. Answer the easy questions first, as this helps you relax (if such a thing is possible when sitting an exam). It is hoped that some of the prospective candidates would have benefited from Daphne's talk.

A presentation on Skin Prick Testing and the recommendations and contra-indications as per the ASCIA 2008 working party were discussed by Jane Kendall. Jane is the Collection Services Manager for Medlab Central and she is based in Palmerston North. The main indicators for the test include rhinitis and asthma. It is important that the patient's skin is healthy and that the patient is co-operative and that he/she is able to cease antihistamines for a period prior to the testing. There are safety concerns if the patient is a baby or infant, the patient has an unstable asthma, is a pregnant woman, or is on beta-blockers. There are also various drugs that can interfere with the test, obviously antihistamines, but also some antidepressants, over-the-counter cold and flu remedies and migraine prophylaxis. Results can also be variable; factors include a recent anaphylaxis reaction to a drug/product or chronic renal failure. An interesting presentation highlighting the importance of asking the right questions regarding medicines being used.

Our next speaker was Kristen Kelly, the Quality Manager at LabPlus. Her topic was NZS/ISO15189 and how it relates to Specimen Services and Phlebotomy. Many laboratory workers

get confused about the ISO standard and IANZ, and often people incorrectly refer to the ISO standard as the IANZ standard. This is incorrect. IANZ accredit against this standard, but it is not an IANZ standard, it is an international standard. Kristen selected various clauses from the standard, in particular section 5, Technical requirements, and discussed how they related to the work we do in our areas. I think as a result of Kristen's talk, there are more staff aware of the reasons why we have to do certain things a particular way, and it's not just because the boss says so! For example, the laboratory shall monitor the transport of samples to the laboratory to ensure they arrive within a suitable timeframe, the final report shall indicate if samples are compromised (e.g. not collected on ice), the signature of the person taking responsibility must be traceable to the request form, sample aliquots shall be traceable to the original primary sample etc

Brenda Parshotam is the Director of Life Impact Strategies and the trustee of Life Impact Trust. Her talk entitled "Take back your Life – Accountable Communication" focused on clear communication and accountability. Taking away the "wiggle room" and taking ownership of your results and empowering others along the way. She talked about the importance of accepting responsibility for the outcome of our choices and being able to take ownership of both our life successes and our life failures. Taking ownership was about "owning our own results" versus evading responsibility for them. The questions were asked of us, am I a slacker, a taker, and do I fail to do my best? Do I follow through on every single promise made? Clarity in communication takes time, it might mean more work, it can even mean loss of face, and asking for a specific commitment may appear aggressive, but an unambiguous agreement might lead to a better deal. A very interesting, thought provoking presentation.

The final speaker of the day was Cathy Bridsen, the Manager of Specimen Services at the PathLab in Hamilton. Cathy's talk was on "Pre-analytics. The Installation of a Beckman Coulter Total Automated System." This new instrumentation and tracking system was evaluated and it showed the key elements that are necessary to get it right. It doesn't always happen first time and requires commitment to evaluating the current process, mapping the work flow, looking for bottlenecks, wastage, steps prone to error and what standardization is necessary e.g. standardization of specimen tube heights. As in all major projects technical support and training requirements are crucial to success. Adjustments have been made to the process but the Beckman Coulter has been successfully implemented and PathLab wouldn't be without it!

The last segment of the program was a question and answer segment that had a panel of "experts" answer questions that had been submitted by the participants on the day. The questions covered a diverse range of subjects from practical phlebotomy procedures, collection on ice (using a slurry of ice),tourniquets use (frequency of cleaning tourniquets vs. disposable tourniquets), isolation of patients and MRSA transmission, venesections (blood pressure cuff use, patient position), in-house training for medical and nursing staff, glove wearing & use of the customer feedback form.

I would like to thank the Presenters for giving up their time on a sunny Saturday, the Chairpersons for their introductions, and the panel of experts who took centre stage for a time in the spotlight. Thank you also to the Northern Region NZAP group members who organised the Presenters and provided us with a great range of interesting topics.

I encourage others to put their hands up and assist with the organisation of a workshop at the NZIMLS Conference in Blenheim on 18th August 2009.

Gerry Heta
NZAP and Specimen Services Co-ordinator

Laboratory Medicine Puzzles

An unusual bleeding disorder

A 16 year-old boy died with a huge haematoma of his leg and abdomen. This was the final occurrence of a life long series of bleeding episodes, which occurred following trauma requiring hospital treatment on more than 50 occasions. Laboratory investigations identified a prolonged bleeding, clotting and prothrombin times, which indicated the presence of a very potent inhibitor of the thrombin-fibrinogen reaction in the plasma. Repeated attempt to stabilize his bleeding disorder failed. There was no history of bleeding disorders in his family and all his family members had normal blood coagulation profiles. His protein electrophoresis identified two bands in the alpha-1region, both below the intensity of the normal control. Immunoassay for alpha-1-antitrypsin was 1.2g/L, which rose to 3.6g/L in the final post-trauma episode (reference range 2.0 to 4.0g/L). Protein purification of the alpha-1-band and testing for alpha-1-activity identified that it failed to inhibit elastase but had a 4000-fold increase in antithrombin activity when compared with normal alpha-1-antitrypsin. Sequencing of the protein identified one amino acid change compared to normal; a methionine was substituted for and arginine at position 358.

Questions

1. What is the normal role of alpha-1-antitrypsin in the body?
2. Comment on the significance of the single amino acid change, why is the mutation at this position so important?
3. The protein electrophoresis showed the double alpha-1-band was low in the quiescent phase but both rose significantly during a crisis. Comment on the type of response this would indicate.
4. Why would the abnormal band initiate bleeding?

An enzyme defect

A young African male presents at a sickle cell outpatients clinic complaining of tiredness, shortage of breath and has a slightly enlarged spleen. He recalls that following a weekend party about two weeks earlier that he frequently took headache tablets containing aspirin, phenacetin and codeine, and felt really ill with some blood appearing in his urine. He stopped the tablets and after a couple of days in bed felt much better, but tired. As he was of African descent some friends suggested that he should be 'checked out' for sickle disease. A sickle cell screen revealed no abnormality and his blood film showed nothing remarkable, but his haemoglobin was 120g/L (reference range 130 to 180g/L). As no obvious abnormality was found he was told to attend outpatients clinic in a months time. Three days after putting mothballs (naphthalene) around his house he feels lethargic and has blood in his urine. On admission to hospital his haemoglobin is 100g?L and he is jaundiced. The blood film shows spherocytes and fragmented red cells but no Heinz Bodies. Measurement of red blood cell glucose-6-phosphate dehydrogenase activity gives a result of 3.9IU/gHb (reference range 5.5 to 7.8IU/gHb.

Questions

1. What is the consequence of low red blood cell glucose-6-phosphate dehydrogenase?
2. What are Heinz Bodies and why do they form?
3. What is the biochemical relationship between glucose-6-phosphate dehydrogenase activity and reduced glutathione?
4. Why is reduced glutathione so important in the red cell?
5. This is an inherited condition, what is the mode of inheritance?
6. If this man has children with a woman who is a carrier for this disorder, predict the outcome for their children.

The sausage eating competition

A healthy 18-year old male entered a sausage eating competition following a bet with his friends that he could easily win the competition. After winning the competition by eating 30 barbecued sausages in 30 minutes he felt light-headed and then collapsed. No heart abnormalities were detected, no evidence of epilepsy and he had not been drinking alcohol as he wanted to eat as many sausage as possible. The major observation while he was unconscious was peripheral cyanosis.

Questions

1. What is peripheral cyanosis?
2. Sausages may contain nitrates, why are they used?
3. What is the interaction with nitrates and haemoglobin?
4. What is the significance of the reaction product between nitrates and haemoglobin?

The death of a child

A 14 month-old West Indian child suddenly developed a chest infection. Her condition deteriorated rapidly and she died in hospital. Laboratory reports indicated a pneumococcal infection. At post-mortem examination a slightly enlarged liver and spleen were noted. Other laboratory results indicated anaemia (haemoglobin 80g.L-1) and an elevated total white cell count of 18000 x 106L-1. An examination of the blood film identified increased reticulocytes and the presence of nucleated red blood cells. The red blood cells were reported as being hypochromic and there was an excess of abnormal (elongated) forms.

Questions

1. What was the underlying disease that precipitated the infection and the child's death?
2. What tests would you recommend to confirm the presumptive diagnosis?
3. There is a single structural difference in the normal and abnormal protein, which is responsible for this disease. What is it and why does it cause red cell abnormalities and anaemia?
4. This disease is normally considered to be a genetic disease in African populations? Do you have any suggestions why it might also be prevalent in West Indian populations?

Answers on page 64

Journal questionnaire

Below are 10 questions based on articles in the August 2009 issue of the Journal. Read the articles fully and carefully, most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site. Make sure you supply your correct email address and membership number. It is recommend that you write your answers in a word doc and then cut & paste your answers on the web site. A number of problems have been reported regarding the submission of the questionnaire in the past. While these are reducing, one major issue still remains. The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try resubmitting from a computer or system using IE Explorer.

You are reminded that to claim valid CPD points for successfully completing the Journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process.

The site will remain open until Friday 13th November 2009. You must get a minimum of 8 questions right to obtain 5 CPD points.

Journal questions

1. How is bone mineral density maintained in the able-bodied population.
2. Which bone markers were measured in the paper by Jones and Legge and by what method principles.
3. What was a significant limitation in the study by Jones and Legge and what did this limitation not allow.
4. In the study by Jones and Legge what evidence does their results present and what further work do they propose.
5. What is generally the gold standard for the management of HIV patients.
6. What can cause anaemia in HIV patients.
7. What determines the antibacterial property of human urine.
8. How does adrenocortical carcinoma usually present in women and what does this suggest.
9. Cortisol-producing adrenal adenoma is indicated by?
10. What do molecular studies of adrenocortical tumour cells show.

Questions and answers for the November 2008 Journal questionnaire

1. What biological phenomena does laminin participate in.
   Adhesion, migration, cellular differentiation and maintenance of the cytoskeleton.
2. At which anatomical site does laminin deposition determine the formation of a true basement membrane along sinusoids and what is this phenomenon called.
   Along the fibers of septal fibrosis and subendothelial sinusoids. This phenomenon is called capillarization of Disse's space.
3. What were the sensitivities, specificities, positive predictive values and negative predictive values for serum laminin before treatment and after treatment.
   Before treatment 96.8%; 80%; 93.7% and 88.8%.
   After treatment 83.9%; 80%; 92.8% and 61.5%.
4. What mechanisms are proposed for the elevation of serum laminin concentrations in chronic hepatitis patients.
   Increased production of laminin in the liver and a lack of degredation of laminin by liver endothelial cells.
5. By what mechanism do the authors think that the gradual decrease in serum laminin after the beginning of treatment occurs.
   Possibly treatment of liver fibrosis causes the liver endothelial cells to regenerate and new endothelial cells degrade laminin.
6. What was the average ratio of Sysmex to DM96 platelet counts and by what percentage and in absolute number does the DM96 overestimate the platelet count.
   Average ratio: 0.86; percentage: 80%; absolute number: about 66 platelets.
7. To what do the authors attribute the consistent overestimation of the DM96 platelet count to and what do they propose to make the DM96 platelet estimation very close to the Sysmex platelet count.
   The platelet estimation factor being set to high. A change from 10 to 0.86.
8. What did the authors consider the advantage and major disadvantage of the DM96 to be.
   Advantage: the overlay grid which aided counting. Major disadvantage: poor quality image.
9. What did the authors state what the recurrent source of error was.
   The total number of cells present in the reclassification differential.
10. What did the authors conclude that the results of their study have demonstrated.
    Results from a 6-part differential performed by the DM96 is similar to results obtained by a manual differential. Microscopy and the trained morphologist still occupy an important place in the haematology laboratory.

Journal article questionnaire Haematology SIG

Article: "Quality counts: New parameters in blood cell counting"

C.Briggs

International Journal of Laboratory Hematology, 2009, Vol 31, Issue 3. p 277-297.

Questions

1. Name five recently introduced complete blood count parameters.
2. What is IRF (immature reticulocyte fraction) used for?
3. Manual reticulocyte counts are imprecise and coefficients of variation between operators have been reported to be as high as 50%. True or False
4. Why are automated reticulocyte counts more precise then manual methods?
5. Different reagents can be used to stain reticulocyte RNA. Match column B with column A:

A		B	
1.	Abbott analyzer	1.	Fluorescent polymethine dye
2.	Horiba Medical Pentra	2.	Fluorescent reagent
3.	Beckman Coulter	3.	New methylene blue stain
4.	Siemens instruments	4.	Thiazole Orange
5.	Sysmex	5.	Oxazine 750

6. When is the IPF (immature platelet fraction) high?
7. Which analyzer has the IPF parameter available?
8. What is the clinical utility of IPF (immature platelet fraction)?
9. What does Ret-He stand for?
10. Define hepcidin.
11. Name four microangiopathies.
12. What is the sensitivity and specificity for automated detection of fragmented RBC?
13. What are two causes of false positive results for RBC fragments?
14. The Abbot immunological method is the most accurate on the samples with what platelet count?
15. What Sysmex parameter represents the internal neutrophil structure?
16. The number of cells falling above the 12 fl threshold divided by the total number of platelets is called:
17. What does an increased ESR reflect?
18. When and where did the British Committee for Standardisation in Haematology introduce interlaboratory quality control?
19. What parameters were initially focused on in the BCSH program?
20. Whose responsibility is it to ensure that there is IQC material available with assigned values for all reportable parameters?

Questions compiled by Helen Kazey, Haematology section, Lab Plus, Auckland Hospital, Auckland.

Contact details for a copy of the journal; Ph (09) 307 4949, ext: 7570. e-mail:helenk@adhb.govt.nz

Answers on page: 65

Med-Bio Journal Award

med·bio

Med-Bio, a division of Global Science & Technology Ltd. offers an award for the best article in each issue of the New Zealand Journal of Medical Laboratory Science. All financial members of the NZIMLS are eligible. The article can be an Original, Review or Technical Article, a Case Study or a Scientific Letter. Excluded are Editorials, Reports, or Fellowship Treatises. No application is necessary. The Editor and Deputy Editor will decide which article in each issue is deemed worthy of the award. If in their opinion no article is worthy then no award will be made. Their decision is final and no correspondence will be entered into.

Winner of the Med-Bio Journal Award for the April 2009 issue was Anthea Povall, Haematology, Canterbury Health, Christchurch for her article "Estimated platelet and differential leukocyte counts by microscopy, Sysmex XE-2100 and CellaVisionô DM96". N Z J Med Lab Sci 2009; 63 (1): 3-10.

NZIMLS Journal Prize

Council of the NZIMLS has approved an annual Journal prize for the best case study accepted and published in the Journal during the calendar year. The prize is worth $200.

Case studies bring together laboratory results with the patient's medical condition and are very educational. Many such studies are presented at the Annual Scientific Meeting, SIG meetings, and the North and South Island Seminars, yet are rarely submitted to the Journal for wider dissemination to the profession. Consider submitting your case study presentation to the Journal. If accepted, you are in consideration for the NZIMLS Journal Prize and will also earn you CPD points. Please contact the Editor or any Editorial Board Member for advice and help. Contact details are on the NZIMLS web site (www.nzimls.org.nz) as are instructions to authors.

No formal application is necessary but you must be a financial member of the NZIMLS during the calendar year to be eligible. All case studies accepted and published during the calendar year (April, August and November issues) will be considered. The Editor, Deputy Editor and the President of the NZIMLS will judge all eligible articles in December each calendar year. Their decision will be final and no correspondence will be entered into.

Blood cell morphology course

Students, staff and friends at the Certificate presentation at the conclusion of the haematology course.

The PPTC'S commitment to teaching and training involves a broad spectrum of educational programmes including training courses held at its centre in Wellington. A four week course in Haematology and Blood Cell Morphology was held in April/May of this year and eight students from throughout the Pacific region attended. Included were Tavite Nifai ' Ete'aki from Vava'u Tonga, Kiaman Raurenti from Kiribati, Mine Kojet from the Marshall Islands, Theresa Tatuava from the Cook Islands, Karma Tenzin from Bhutan, George Pakoa and Malau Kalo from Vanuatu and Herbert Johnny from the Federated States of Micronesia. Phil Wakem, our newly appointed Programme Co-ordinator and Haematology lecturer, presented a comprehensive learning programme over the four week period.

The course provided students with guidelines for the objective microscopic evaluation of white cells, red cells and platelets in both health and disease. The students also learnt to correlate the blood film findings with results obtained from manual and/or automated methods for red cell, white cell and platelet parameters. Overall the course was designed to take the students on a haematological journey beginning with the principles of microscopy, travelling comprehensively both the normal and pathological pathways of Haematology and concluding with the final staining, examination and interpretation of the blood film. From the feedback that we have already received from students, it is obvious that they learnt a great deal during this course and they are already putting these new skills to use in the diagnosis of haematological disorders.

We were also fortunate to have Marilyn Eales return for the last week of the course and assist Phil in delivering the laboratory practicals. Marilyn has had a very long and dedicated association with the PPTC as a consultant, Haematology lecturer and member of the Board representing the NZIMLS. She is always so willing to share her wealth of knowledge and experience with the students and we were very grateful she was able to return to Wellington at this time.

Ms Megan McCoy from NZAID was the guest at the Certificate presentation at the conclusion of the course and as well as presenting the students with their certificates, spoke about NZAID's commitment to Pacific Island countries in assisting them in working towards better health systems for their people and the vital role that laboratories play in this.

Courses for the rest of 2009

We have two further courses scheduled for 2009 these are Microbiology scheduled for 14 September to 9 October and Blood Bank Technology scheduled for November.

Country visits

Tonga: Phil has just returned from a week's visit to Tonga where he conducted a quality assurance audit at the Viola Hospital Laboratory in Nuku'alofa. This laboratory has had a Quality Assurance programme in place for some years and the PPTC acts in an advisory and supportive role in terms of guiding the laboratory in meeting required quality standards. Phil is slowly coming to terms with the warmer climate patterns of the Pacific nations and has threatened to wear a lavalava and jandals if it gets much hotter. That will be a sight to behold.

Samoa: Recently John Elliot visited the National Laboratory in Apia, Samoa and took part in a very successful workshop organised by Samoa Red Cross on Voluntary Non-renumerated Blood Donors. While there he also had talks with laboratory and National Health Service management regarding the development of the laboratory's quality management programme.

Pihoa: In March both John and Phil attended the PIHOA [Pacific Islands Health Officers Association] Laboratory Network Meeting in Guam. It was great to meet laboratory staff from the North Pacific laboratories and inform them of enhancements in the PPTC's programmes, discuss with them laboratory quality management and present graduates of the DipMLT course provided by the PPTC through POLHN with their diplomas.

Both John and Phil are planning on visiting a number of other laboratories during the rest of this year and we look forward to meeting and working with you our laboratory colleagues in giving any assistance we can to help you improve your laboratory and the service you give to the people of your country.

Abstracts of articles in the British Journal of Biomedical Science

Elliott K, Hamilton PW, Maxwell P. Fluorescence (FISH) and chromogenic (CISH) in situ hybridisation in prostate carcinoma cell lines: comparison and use of virtual microscopy. *Br J Biomed Sci 2008; 65(4): 167-71.*

Chromogenic in situ hybridisation (CISH) has become an attractive alternative to fluorescence in situ hybridisation (FISH) due to its permanent stain which is more familiar to pathologists and because it can be viewed using light microscopy. The aim of the present study is to examine reproducibility in the assessment of abnormal chromosome number by CISH in comparison to FISH. Using three prostate cell lines--PNT1A (derived from normal epithelium), LNCAP and DU145 (derived from prostatic carcinoma), chromosomes 7 and 8 were counted in 40 nuclei in FISH preparations (x100 oil immersion) and 100 nuclei in CISH preparations (x40) by two independent observers. The CISH slides were examined using standard light microscopy and virtual microscopy. Reproducibility was examined using paired Student's t-test ($P<0.05$). Reproducibility between observers was good for both FISH and CISH. No significant differences in chromosome count were seen between the techniques. Chromosomes 7 and 8 showed disomic status for each cell line except LNCAP, which proved to be heterogeneous (disomic/aneusomic), particularly for chromosome 8. Virtual microscopy proved to be easy to use and gave no significant differences from standard light microscopy. These results support the hypothesis that there is no significant difference between FISH and CISH techniques.

Zhang FB, Sui LH, Xin T. Correlation of Bmi-1 expression and telomerase activity in human ovarian cancer. *Br J Biomed Sci 2008; 65(4): 172-7.*

This study investigates the correlation between the oncoprotein Bmi-1 and telomerase activity in ovarian cancer. A real-time polymerase chain reaction (PCR) method is used to detect the messenger RNA (mRNA) expression of Bmi-1 protein in 47 ovarian epithelial cancer cases, and immunohistochemistry is used to detect Bmi-1 protein expression in the tissues. A modified telomeric repeat amplification protocol (TRAP) is used to detect telomerase activity. Western blotting is used to detect the expression of telomerase hTERT in the tissues studied. Compared to normal ovarian epithelial tissue, Bmi-1 protein in the 47 ovarian epithelial cancer cases showed higher expression and was related to pathological grade and clinical stage. Significantly higher Bmi-1 levels were found among different clinocopathological types of the cancer ($P<0.05$). Grade G3 cases expressed Bmi-1 at a higher rate (93.10%) than did grade G2 cases (61.11%). Expression in phase II and phase III cases was lower (66.67%) than in phase IV (92.31%). In ovarian epithelial cancer tissues, 87.23% (41/47) cases demonstrated positive telomerase activity, whereas no activity was observed in normal tissues. The majority (90.24%) of specimens with positive telomerase activity showed high Bmi-1 expression levels. Spearman correlation analysis indicated that expression of Bmi-1 protein correlated positively with elevated telomerase activity. Bmi-1 protein is highly expressed in ovarian epithelial cancer tissues, and expression correlates with histological grade and clinical phase. Elevated Bmi-1 expression correlates closely with increased telomerase activity and plays a significant role in the pathogenesis of ovarian cancer.

Saxena S, Gomber C. Comparative in vitro antimicrobial procedural efficacy for susceptibility of Staphylococcus aureus, Escherichia coli and Pseudomonas species to chloramphenicol, ciprofloxacin and cefaclor. *Br J Biomed Sci 2008; 65(4): 178-83.*

The present study assesses the reliability of the in vitro susceptibility tests E-test, disc diffusion and Alamar blue-based microbroth dilution assays for evaluating the efficacy of chloramphenicol, ciprofloxacin and cefaclor against clinical and reference isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas spp. for use in empirical therapy. Ciprofloxacin showed 82% agreement between E-test and microbroth dilution by the visible dye reduction method against all organisms. This figure was 67% for cefaclor and 60% for chloramphenicol. E-test and microbroth dilution showed excellent correlation against S. aureus with all three antibiotics; however, correlation was not observed in Pseudomonas spp. between E-test and microbroth dilution by the percentage dye reduction method. E. coli did not show significant correlation, indicating the presence of heteroresistance. Chloramphenicol, being bacteristatic in nature, did not show clear agreement between the susceptibility test methods used. This study indicates that E-test provides an indication of minimum inhibitory concentration (MIC) of a panel of antibiotics against clinical isolates; however, microbroth dilution (dye reduction) is the most sensitive method for the determination of MIC due to intracellular enzymatic reduction of the dye to formazan, which only occurs in viable organisms. The study highlights the need for harmonisation between E-test and microbroth dilution methods in clinical trials of new antibiotics and in monitoring the drug resistance patterns in community and healthcare settings.

Sousa C, Henriques M, Teixeira P, Oliveira R. Reduction of Staphylococcus epidermidis adhesion to indwelling medical devices: a simple procedure. *Br J Biomed Sci 2008; 65(4): 184-90.*

The present study aims to find a method to reduce Staphylococcus epidermidis adhesion to acrylic and silicone--two materials used commonly in medical devices--by heparin and gentian violet surface preconditioning. Different periods of heparin preconditioning are studied to evaluate the influence of preincubation time on the reduction of bacterial adhesion. A two-hour period was chosen and applied in the adhesion assays with either heparin or gentian violet. Squares of the materials with adherent cells were also analysed by scanning electron microscopy (SEM). Results of adhesion assays showed a significant reduction (53-90%, $P<0.05$) in bacterial adhesion to silicone and acrylic after precontact with the conditioning substances. No statistical differences ($P>0.05$) were found between the extent of adhesion on silicone coupons precontacted either with heparin or gentian violet for each of the strains tested. On acrylic, heparin was more efficient ($P<0.001$) in reducing S. epidermidis IE186 adhesion than was gentian violet (85% and 53% reductions, respectively). Therefore, immersion of acrylic and silicone in heparin or gentian violet may constitute a simple and effective method by which to reduce S. epidermidis adhesion to medical devices.

Sharma R, Cooke RP, Ratcliffe JG. Detection of ESBL bacteria from clinical specimens: evaluation of a new selective medium. *Br J Biomed Sci 2008; 65(4): 191-4.*

Selective screening media for extended-spectrum beta-lactamase (ESBL)-producing bacteria are needed to guide antibiotic therapy and institute appropriate infection control measures. This study evaluates a selective cefpodoxime-incorporated chromogenic agar (CCA) medium for the detection of ESBLs from clinical specimens. The medium was formulated specifically for this

study. For all culture-positive urine samples and wound swabs from intensive care unit (ICU) patients, CCA was compared with standard laboratory testing procedures and HPA/BSAC guidance on ESBL detection. The CCA medium was also evaluated for ESBL faecal carriage from patients on ICU and the haematology ward. These patients had no prior evidence of colonisation or infection with ESBL-producing bacteria. All ESBL isolates underwent minimum inhibitory concentration (MIC) testing to cefpodoxime. The Miles and Misra method and the ecometric methods were used to quality control the microbiological performance of the CCA medium, which proved satisfactory. A total of 750 specimens were examined (690 urines, 40 faeces, 20 wound swabs). From urine cultures, 92 suspect colonies were followed up. Eighteen were cefpodoxime-resistant on routine disc testing and all were confirmed subsequently as ESBL-positive. Conventional laboratory methods identified only two urinary ESBLs. Wound cultures revealed two suspect colonies, both of which were ESBL-positive and were also detected by routine methods. Faecal samples produced 10 suspect colonies, six of which were ESBL-positive. All ESBLs had cefpodoxime MICs >10 mg /L (75% were >256 mg/L). Thus, primary conventional culture methods cannot be relied upon to detect suspect ESBL-producing bacteria.

Matsuda M, Shigematsu M, Tazumi A, Sekizuka T, Takamiya S, Millar BC, Taneike I, Moore JE. Cloning and structural analysis of the full-length cytolethal distending toxin (cdt) gene operon from Campylobacter lari. *Br J Biomed Sci 2008; 65(4): 195-9.*

Polymerase chain reaction (PCR) amplicons (approximately 2.5 kbp) encoding a cdt gene operon and two partial and putative open reading frames (ORFs) were identified in six urease-negative (UN) Campylobacter lari isolates using a new PCR primer pair constructed in silico. Three closely spaced and putative ORFs for cdtA, cdtB and cdtC, two putative promoters and a hypothetically intrinsic p-independent transcription terminator were found in the operon. Each ORF commenced with an ATG start codon and terminated with a TGA stop codon for cdtA and cdtB and a TAA for cdtC. Interestingly, an overlap of four nucleotides was detected between cdtA and cdtB and the non-coding region of six base pairs occurring between cdtB and cdtC. The start codons for the three cdt genes were preceded by Shine-Dalgarno sequences. Although nucleotide sequence differences were identified at seven loci in the cdtA gene, six in cdtB and two in cdtC among the seven isolates (including C. lari RM2100), no polymorphic sites occurred in the putative promoters, hypothetically intrinsic transcription terminator and the three ribosome binding sites among the seven isolates. All nine amino acid residues specific for both Escherichia coli cdtB and mammalian DNase I were completely conserved in the cdtB gene locus in the 26 C. lari isolates, as well as in C. jejuni and C. coli. No PCR amplicons were generated with urease-positive thermophilic campylobacters (UPTC; n=10) using the primer pair.

Hancock JT. Cell signalling is the music of life. *Br J Biomed Sci 2008; 65(4): 205-8.*

Cell signalling is an immensely important topic in biological and biomedical sciences, and one which has an ever-increasing literature. As more and more is known about it, and more components are discovered, it is getting harder and harder to visualise how it all might work to create an holistic mechanism in the cell. To achieve a better understanding of a complex issue such as this, it is often useful to use an analogy which is familiar to the researcher to encourage better understanding. In this essay it is suggested that music, and the instruments used to produce it, can be used as such an analogy. Various elements and issues in cell signalling are discussed and musical comparisons are made. Clearly, the true understanding of cell signalling will come from systems biology and mathematical modelling, but it is proposed that this analogy might prove useful. The phrasing used may be considered a little loose and flamboyant for a scientific topic of such importance, but it is hoped that the discussion will not only be interesting but might also be useful in fostering debate and facilitating teaching in this area of molecular biology.

Blann AD, Nation BR. Good analytical practice: statistics and handling data in biomedical science. A primer and directions for authors. Part 1: Introduction. Data within and between one or two sets of individuals. *Br J Biomed Sci 2008; 65(4): 209-17.*

The biomedical scientist is bombarded on a daily basis by information, almost all of which refers to the health status of an individual or groups of individuals. This review is the first of a two-part article written to explain some of the issues related to the presentation and analysis of data. The first part focuses on types of data and how to present and analyse data from an individual or from one or two groups of persons. The second part will examine data from three or more sets of persons, what methods are available to allow this analysis (i.e., statistical software packages), and will conclude with a statement on appropriate descriptors of data, their analyses, and presentation for authors considering submission of their data to this journal.

Wren MW, Sivapalan M, Kinson R, Shetty NR. Laboratory diagnosis of clostridium difficile infection. An evaluation of tests for faecal toxin, glutamate dehydrogenase, lactoferrin and toxigenic culture in the diagnostic laboratory. *Br J Biomed Sci 2009; 66(1): 1-5.*

Faecal samples from 1007 patients suspected of having diarrhoea caused by Clostridium difficile infection are investigated for the presence of toxins A and B and for the presence of C. difficile-specific glutamate dehydrogenase (GDH). Toxigenic culture is performed on all samples and is used as the 'gold standard' for the purpose of the study. A marker for intestinal inflammation, faecal lactoferrin, is used on any samples that give a positive result in any of the above tests. Part of the study also involves an assessment of six commercial toxin kits to detect the presence of C. difficile toxins in faecal samples. This study revealed that the commercial toxin detection kits used can give rise to false-positive and false-negative results and that all demonstrated poor sensitivity when compared to the gold standard of toxigenic culture. Testing of faecal samples for GDH can be useful as a negative screening method as the results of this test show high correlation with culture. Faecal toxin testing can then be performed on all GDH-positive samples (GDH positivity is independent of toxigenicity in strains of C. difficile). The combined use of GDH and toxin testing, coupled with toxigenic culture, revealed that some patients with diarrhoea who harboured toxigenic strains of C. difficile were faecal toxin-negative. Lactoferrin appears to be a useful marker for the presence of inflammatory diarrhoea.

Bamber AI, Cunniffe JG, Nayar D, Ganguly R, Falconer E. Effectiveness of introducing blood culture collection packs to reduce contamination rates. *Br J Biomed Sci 2009; 66(1): 6-9.*

Contaminated blood cultures result in a significant waste of healthcare resources and can lead to inappropriate antibiotic therapy. Practitioners have taken measures to reduce contamination rates. These include thorough skin disinfection, effective hand decontamination, introduction of a standardised approach to collection, and the introduction of blood culture collection packs (BCCP). This study aims to assess the impact of introducing BCCP and staff training on the rate of contamination. The study demonstrated that contamination rates are greatest

in high patient throughput units where practitioners are under most pressure. The introduction of blood culture packs and staff training has reduced contamination rate significantly from 43% to 25% of the total number of positives, equating to an overall reduction of 42%. Thus, there is a demonstrable benefit in the purchase of commercially produced blood culture packs and the investment in staff training.

Oyedeji KS, Smith SI, Coker AO, Arigbabu AO. Antibiotic susceptibility patterns in Helicobacter pylori strains from patients with upper gastrointestinal pathology in western Nigeria. *Br J Biomed Sci 2009; 66(1): 10-3.*

A total of 186 Helicobacter pylori isolates and 532 gastric biopsies recovered from 532 patients with varying degrees of gastroduodenal pathology are subjected to in vitro antibiotic susceptibility testing using the disc-diffusion method, Etest (MIC breakpoints) and molecular testing using the polymerase chain reaction (PCR). In the isolates studied, antibiotic resistance was as follows: piperacillin (72%), amoxicillin (66%), erythromycin (78%), tetracycline (100%) and metronidazole (95%). All isolates were sensitive to ofloxacin, ciprofloxacin and norfloxacin. None of the 245 amplicons (positive for H. pylori) from the biopsies were digested with the Bbs1 and Bsal restriction enzyme used in the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, showing sensitivity to clarithromycin. However, a 238 bp fragment from H. pylori chromosomal DNA (corresponding to the quinolone resistance determining region [QRDR]) of the gyrA gene was amplified successfully. Twelve (4.9%) of the 245 strains studied had the described mutation at position 91, from asparagine (Asn) to glycine (Gly). The study showed that all the H. pylori strains were sensitive to clarithromycin and ciprofloxacin. It also highlighted PCR as a potential tool for faster diagnosis and determination of antibiotic susceptibility (within 24 h) of H. pylori from biopsies and/or isolates recovered from peptic ulcer and gastritis patients.

Kriushnapriya S, Malathy NS, Shamitha Begum A, Baskaran AC, Appalaraju B, Mani K, Kandhaswamy KA. Anti-MRSA activity of aldehyde Schiff base N-aryl thiosemicarbazones. *Br J Biomed Sci 2009; 66(1): 14-9.*

Eight different newly synthesised aldehyde Schiff base N-aryl thiosemicarbazones, differing in R, R' groups, are tested on 25 clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) and a standard strain. Antibacterial activity was carried out by a well-diffusion method in concentrations of 15-500 microg/well. Compounds 1, 2, 3, 4 and 6 showed good inhibition of MRSA. Increasing concentration of the test compounds enlarged the inhibition zone. Determination of minimum inhibitory concentration (MIC) was carried out using a dilution susceptibility test in concentrations of 4-512 microg/mL of the medium. The lowest MIC value (16 microg/mL) was produced by compound 4.

Mu HJ, Xie R, Shen YF, Jiang YQ, Zeng YJ. Cadherin-13 in primary and blast crisis chronic myeloid leukaemia: declining expression and negative correlation with the BCR/ABL fusion gene. *Br J Biomed Sci 2009; 66(1): 20-4.*

Expression of the CDH13 gene in chronic myeloid leukaemia (CML) patients at different clinical stages, and its relationship with the BCR/ABL fusion gene, is investigated. Expression of the CDH13 gene and the BCR/ABL fuse gene is investigated in peripheral blood from 30 healthy adults, 25 primary CML patients and 25 CML patients in blast crisis using the EvaGreen real-time reverse transcriptase polymerase chain reaction (RT-PCR). Results showed that BCR/ABL fusion gene expression in the blast crisis CML patients was 4.72-fold higher than that in patients with primary CML. Expression of CDH13 mRNA in primary and blast crisis CML patients was lower than in the healthy adults, reduced 64% and 75%, respectively. Expression of CDH13 showed a negative correlation with the BCR/ABL fusion gene. The data indicate that the decline of CDH13 expression accompanied the different clinical stages of CML and probably was involved in over-expression of the BCR/ABL fusion gene.

Devraj JP, Shankarkumar U, Ghosh K. Increased frequency of HLA-B7 among B27-negative seronegative spondarthritis patients from Mumbai, western India. *Br J Biomed Sci 2009; 66(1): 25-7.*

Seronegative spondarthritis (SSA) is a group of inflammatory disorders that shares certain clinical features and has a strong association with the human leucocyte antigen (HLA)-B27 allele. Serologically, HLA-B27, HLA-B22, HLA-B7, HLA-B40 and HLA-B42 antigens belong to the HLA B cross-reacting antigen group (CREG). In addition to B27, other B locus antigens are associated with B27-negative American black, Brazilian, French and Chinese SSA patients. Many B27-negative individuals in India have developed SSA with severe clinical and radiological findings. This stimulated the evaluation of the involvement of HLA-B7 CREG antigens among B27-negative SSA patients from western India. A total of 276 SSA patients who were B27-negative and fitted the modified New York criteria for AS and the European Spondyloarthropathy Study Group (ESSG) criteria for spondarthritis from western India were studied and compared with 637 normal, healthy individuals who were B27-negative and of the same ethnic background. A significantly increased phenotype frequency of HLA-B7 (PF = 57.24% vs. 22.44%; $P < 0.001$) and a significant decreased phenotype frequency of HLA-B40 (PF = 18.11% vs. 31.86%; $P < 0.001$) was observed when compared to the controls. These results suggest that HLA-B7 antigen may be associated with B27-negative SSA in patients from western India.

Campo GM, Avenoso A, Campo S, D'Ascola A, Traina P, Calatroni A. Effect of cytokines on hyaluronan synthase activity and response to oxidative stress by fibroblasts. *Br J Biomed Sci 2009; 66(1): 28-36.*

Cytokines such as tumour necrosis factor-alpha (TNFalpha), interferon-gamma (IFNgamma), and transforming growth factor-beta (TGF1beta) modulate hyaluronan synthase (HAS) gene expression and protein activity. The aim of this research is to evaluate the response of HAS gene expression and the related protein synthesis in fibroblasts after treatment with TNFalpha, IFNgamma and TGF1beta and to assess the potential protective effect of increased hyaluronan (HA) synthesis during oxidative stress. In this study, gene expression, protein synthesis, hyaluronan content, cell death, lactate dehydrogenase (LDH) activity, membrane lipid peroxidation and endogenous antioxidant depletion are determined for HAS1, HAS2 and HAS3. Messenger RNA (mRNA) expression and protein formation of the three HAS genes is modulated using different cytokines and various doses and correlated with increased HA synthesis. Protection of fibroblasts from injury induced by exposure to reactive oxygen species was significantly increased by TGF1beta and was associated with increased gene expression and protein formation of HAS1 and HAS2 enzymes synthesising high-molecular-weight HA. It is proposed that specific HAS enzyme activity and HA molecular weight specificity is involved in the protective mechanism.

Moore JE, Watabe M, Millar BC, Rooney PJ, Loughrey A, Goldsmith CE, McMahon MA, McDowell DA, Murphy RG.

Molecular characterisation of the recA locus in clinical isolates of verocytotoxigenic E. coli O157:H7. *Br J Biomed Sci 2009; 66(1): 37-41.*

Molecular epidemiology of verocytoxigenic Escherichia coli O157:H7 is important to help elucidate reservoirs and modes of transmission, particularly between animals and humans. As the recA gene locus is now beginning to gain application in bacterial genotyping schemes, and as it has not been examined previously in E. coli O157 isolates, this study aims to examine potential polymorphic variation as a possible epidemiological marker for the subspecies characterisation of clinically significant verocytotoxigenic E. coli O157:H7. A novel polymerase chain reaction (PCR) assay was designed to target a 638 bp region of the recA gene in E. coli O157 isolates. The PCR amplification of genomic DNA from extracted organisms was able to generate an amplicon of the expected size (approximately 638 bp) for all E. coli O157:H7 examined (n=80), as well as for other non-O157 E. coli and other members of the Enterobacteriaceae including Citrobacter, Hafnia, Shigella, Enterobacter and Providencia. Subsequent restriction fragment length polymorphism (RFLP) and single-stranded conformational polymorphism (SSCP) analyses of these recA amplicons were able to differentiate E. coli O157 from the organisms examined, but were unable to distinguish between 79 isolates of wild-type E. coli O157, suggesting a highly conserved recA gene structure within the local population of organisms examined.

Blann AD, Nation BR. Good analytical practice: statistics and handling data in biomedical science: A primer and directions for authors. Part 2: analysis of data from three or more groups, and instructions for authors.
Br J Biomed Sci 2009; 66(1): 42-6.

Biomedical scientists are bombarded daily by information, almost all of which refers to the health status of an individual or groups of individuals. This article is the second of a two-part review written to explain some of the issues related to presentation and analysis of data. In the first part (Br J Biomed Sci 2008; 65: 209-17) we focused on types of data, and how to analyse and present the data from an individual or from two groups of persons. Here, we will continue with an examination of data from three or more sets of persons, what methods are available to allow this analysis (i.e., statistical software packages), and will conclude with a statement on appropriate descriptors of data, their analyses and presentation, for authors considering submitting their data to this journal.

NZIMLS South Island Seminar 21st March 2009

This year's South Island Seminar (SIS) was held at the Rydges Hotel Christchurch on Saturday 21st March 2009 and with over 180 registrants the popularity of this event was evident. The SIS has always been a great forum for multidisciplinary presentations and an opportunity to network with colleagues from around the country, this year's event was no exception.

Internationally renown Editor in Chief of "The Dark Report" Robert Michel opened the meeting with a thought provoking talk on "The Changing Face of Laboratory Organization and Operations".

Attendees were treated to a series of high quality presentations throughout the day which included a variety of scientific and management papers, also an overseas work experience presentation and another that can only be described as an entertaining motivational speech.

As part of the NZIMLS Council's commitment to promote the profession, the final session ended with the screening of the "Just the Job" promotional DVD on Medical Laboratory Science. This programme was warmly received by the audience and following it's airing on TV2 on Saturday 9th May 2009 should raise the profile of the profession and encourage school leavers to pursue a career in medical laboratory science.

The Bio-Rad Award for best speaker (first or second time presenter) at the SIS was presented to Michael Awadalla for his talk on "MRSA". The NZIMLS awarded the prize for best overall scientific paper to Jill Taylor for her presentation entitled "Microscope to microarray: a molecular cytogenetic case study".

All in all the feedback suggested another very successful SIS and the NZIMLS organisers wish to thank all the presenters and Roche Diagnostics who sponsored the day and contributed towards the overall success of this year's seminar. We look forward to another well attended stimulating South Island Seminar next year.

Ken Beechey
NZIMLS Region 4 Representative

Book review

A Guide to Laboratory Investigations by Michael McGhee Publisher: Radcliff. Fifth edition (2008) 186 pages

The primary purpose for this book is as a guide for general medical practitioners (GP) and covers areas of laboratory medicine the GP is likely to request. It has chapters relating to Haematology, Microbiology, Fertility and Pregnancy Testing, Rheumatology and Immunology, Biochemistry and Miscellaneous (therapeutic drug monitoring, cervical smears, urinanalysis, DEXA scans).

Overall the book provides a relatively quick guide to the interpretation of routine pathology results. Unfortunately there was a mix of SI and non-SI units used in the book, which will not help interpretation. As a basic guide, however the book works well giving information and interpretation on most of the more commonly requested diagnostic pathology tests for the GP to consider when interpreting results.

There were some omissions, which should have been considered. There is no mention of haemochromatosis and an introduction to medical genetics would have been a useful section given the increasing public awareness of this area. In the sections on thrombophilia and haemoglobinopathies there is no reference to the inheritance mechanisms for these disorders, which is essential for families to understand the diseases. Similarly with Glucose-6-Phosphate Dehydrogenase deficiency, it would have been helpful to provide examples of drugs and infections, which may cause haemolytic episodes. An Appendix outlining interpreting results relating to reference ranges (diurnal variation, diet, activity, checking against previous results etc) would provide the user with useful information when considering any results.

The book is well presented and the index is accurate and will be useful reference for its target audience.

Michael Legge
University of Otago, Dunedin

The National Immunohaematology Continuing Education (NICE)

held the 20th meeting on the weekend of 1-3 May 2009.

This was attended by Transfusion people from around NZ and is unique in that all participants present a short paper or display a poster.

Sheryl Khull and Will Perry have attended all 20 NICE weekends. (Photo)

The weekend has been held every year bar one at the Bayview Wairakei Resort Hotel

Winners and highly commended presenters were (Photo from left)

Aous Al-Ibous (AUT student) highly commended for **"Freezing and Recovering Red Cells"**

Joanne Colgan (AUT student) highly commended for **"MICA Antibodies in NZ Renal transplant Patients"**

Zayna Hussein (AUT student) Best first time presenter and winner of the NZIMLS Student Scholarship for her talk **"Pathogen Reduction"**

Belinda Drummond (Nelson Hospital) Inaugural CSL Travel and Conference Award. For her talk **"Case Studies and the Lethal Triad"**

Cecelia Wall (Hutt Valley Hospital) Pharmaco award for the best poster **"Anti-D, Anti-G and the role of Prophylactic Anti-D during Pregnancy"**

Melissa Nelson (NZBS Blood Bank Waikato Hospital) The Overall Best Presentation and Abbott Award for her talk **"Chocolate or Aspirin?"**

We congratulate these presenters and all the presenters who attended the weekend, the judging panels and Industry people who helped to make it - **yet again a NICE weekend**

Raewyn Cameron *co convenor*
Diane Whitehead *co convenor*
Holly Perry *TSSIG convenor*

Laboratory medicine puzzles – answers

An unusual bleeding disorder

1. What is the normal role of alpha-1-antitrypsin in the body?
   Alpha-1-antitrypsin is the major anti-protease in the blood, protecting normal tissues during periods of stress (such as inflammation) from elastase released from activated and degenerating neutrophils. In the lungs it inhibits elastase, preventing destruction of lung elastin.

2. Comment on the significance of the single amino acid change, why is this mutation at this position so important?
   Methionine at position 358 is in the binding pocket for elastase. Conversion of methionine (non-polar, sulphur containing) to arginine (larger, positively charged) alters the specificity of alpha-1-antitrypsin. The single amino acid alteration changed the specificity of alpha-1-antitrypsin from elastase to antithrombin and reduced the boy's thrombin activity. The reduction in thrombin precipitated haemorrhage, which ultimately proved fatal.

3. The protein electrophoresis showed the double alpha-1-band was low in the quiescent phase but both rose significantly during a crisis. Comment on the type of response this would indicate.
   This is an acute phase response. Alpha-1-antitrypsin is an acute phase protein. In the quiescent phase there is no response therefore the bands are low. However, during a crisis there is an up-regulation of both the normal and abnormal alpha-1-antitrypsin. The single amino acid change is sufficient to change electrophoretic mobility, hence the two bands in the alpha – 1 region.

An enzyme defect

1. What is the consequence of low red blood cell glucose-6-phosphate dehydrogenase?
   Low glucose-6-phosphate dehydrogenase (G6PDH) activity means low amounts of NADPH are produced and consequently low amounts of reduced glutathione will be synthesized thereby making the red cell more susceptible to oxidative damage.

2. What are Heinz bodies and why do they form? **Heinz bodies are intracellular precipitates or inclusions (i.e. clumps of denatured proteins) that adhere to the plasma membrane of red cells and stain with basic dyes. Some reasons why Heinz bodies form are: an amino acid substitution in the haem pocket that causes the haem to be displaced resulting in reduced solubility of the haemoglobin; and oxidative haemolysis caused by certain drugs and other compounds (eg naphthalene, aspirin, chloramphenicol) causing precipitation of intracellular proteins which adhere to the inner red cell membrane.**

3. What is the biochemical relationship between glucose-6-phosphate dehydrogenase and reduced glutathione?
   G6PDH catalyses the following reaction:
   Glucose-6-phosphate + NADP+ → 6-phophogluconolactone + NADPH

   Glutathione reductase then catalyses:
   NADPH + oxidised glutathione → NADP+ + reduced glutathione

4. Why is reduced glutathione so important in the red cell?
   Reduced glutathione (GSH) protects against oxidative damage, maintains haemoglobin iron in the ferrous form, maintains the integrity of the red cell membrane and is involved in detoxification.

5. This is an inherited condition, what is the mode of inheritance?
   X-linked recessive.

6. If this man has children with a woman who is a carrier for this disorder, predict the outcome for their children.
   The man is affected (X*Y) and the woman is an unaffected female carrier (X*X). Therefore, 25% affected males (X*Y), 25% unaffected males (XY), 25% heterozygote carrier females (X*X), 25% homozygote affected females (X*X*).

The sausage eating competition

1. What is peripheral cyanosis?
   Lack of oxygen to the peripheral tissues. The patient's skin appears to have a slaty, grey-blue colour.

2. Sausages may contain nitrates, why are they used?
   Food preservative.

3. What is the interaction with nitrates and haemoglobin?
   They oxidise the iron in haemoglobin from the ferrous (Fe2+) to the ferric (Fe3+) state, forming methaemoglobin.

4. What is the significance of the reaction of the reaction product between nitrates and haemoglobin?
   The nitrates in the sausages oxidised the patient's haemoglobin to methaemoglobin, which cannot carry oxygen. This reduces the supply of oxygen to the tissues (hence the cyanosis) and the brain (hence the unconsciousness).

The death of a child

1. What is the underlying disease that precipitated the infection and the child's death?
   Sickle cell anaemia (HbS).

2. What tests would you recommend to confirm the presumptive diagnosis?
   Perform a blood film and possibly a wet-prep to look for sickle cells, ESR will be high, haemoglobin electrophoresis, DNA analysis for the mutation.

3. There is a single structural difference in the normal and abnormal protein, which is responsible for the disease. What is it and why does it cause red cell abnormalities?
   The mutation changes a glutamate to valine at position 6 in the beta-globin chain of haemoglobin. The valine causes a "sticky patch" on the outside of the HbS. In deoxyhaemoglobin S there is a hydrophobic site complementary to the "sticky patch" and so the HbS proteins can stick together. This binding creates long fibrils of HbS in the red cells. In addition there are also abnormalities in the structure of the red cell walls. The abnormal red cells are broken down at a faster rate than they are replaced thereby causing the anaemia.

4. This disease is normally considered to be a genetic disease in African populations. Do you have any suggestions why it might also be prevalent in West Indian populations?
   Movement of genes from Africa with the slave trade and subsequent interbreeding with the West Indian population (who were also slaves) ultimately transferred this disease to an otherwise sickle cell disease free population.

News from the Universities

The AUT annual awards ceremony for the top 2008 graduating students was held on Friday 22nd May. It was a very pleasant evening with the formalities followed by a chance to socialise. The NZIMLS Council attended the function and Robin Allen presented the NZIMLS Prize for the **most outstanding Bachelor of Medical Laboratory Science graduate to Amy Ying Huang.** Amy is now working at Donation Accreditation in New Zealand Blood Service.

Other medical laboratory science prize winners were;

- Matthew Bluck, winning the Fort Richard Laboratories Ltd Prize for the most outstanding BMLS graduate in Medical Microbiology and the OCD Prize for the most outstanding BMLS graduate in Clinical Chemistry
- Dawit Kussale winning the Roche Diagnostics New Zealand Ltd Prize for the most outstanding BMLS graduate in Immunology
- Marrean Theseira, winning the Diamed Prize for the most outstanding BMLS graduate in Transfusion Science
- Pei-Chi Lin, winning the Beckman Coulter Prize for the most outstanding BMLS graduate in Haematology

All these graduates are now either working in our industry, or pursuing post graduate qualifications in medical laboratory science at AUT. Our congratulations to them all.

Answers to the HSIG journal questionnaire

1. Nucleated red blood cells; immature granulocytes; immature reticulocyte fraction; immature platelet fraction; red cell fragments
2. To demonstrate a response to treatment for anaemia and predict engraftment after stem cell transplants.
3. True
4. Automated counts are more precise because of the much higher number of cells being counted.
5. A 1.Abbott analyzers
   2.Horiba medical Pentra
   3.Beckman Coulter
   4.Siemens instruments
   5.Sysmex

   B 2. Fluorescent reagent
   4.Thiazole Orange
   3.Methylene blue stain
   5.Oxazine 750
   1.Fluorescent polymethine dye
6. IPF is high when there is peripheral consumption or destruction of platelets.
7. Sysmex XE series
8. Differential diagnosis of thrombocytopenia, prediction of platelet recovery post transplant or chemotherapy.
9. The reticulocyte haemoglobin equivalent
10. Iron regulatory hormone produced in the liver and acute phase reactant which prevents iron absorption from the gastrointestinal tract.
11. Haemolytic uraemic syndrome; thrombotic thrombocytopenia purpura; disseminated carcinoma; disseminated intravascular coagulation.
12. Sensitivity 100% and specificity 20%.
13. Presence of anisopoikilocytosis and nonschistocyte fragments.
14. Samples with a platelet count of 20x10^9/L or less.
15. Sysmex Neut-X (mean value for side scatter diffraction of the neutrophil population).
16. P-LCR (platelet larger cell ratio).
17. It reflects increase in plasma fibrinogen and other plasma proteins including immunoglobulins.
18. United Kingdom in 1968.
19. Initially they focused on the red blood cell count, MCV, haemoglobin and packed cell volume.
20. Manufacturer's responsibility to ensure that there is IQC material available with assigned values for all reportable parameters.


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