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Editorial

Original articles

Letter to the Editor

Regular features

Editorial:
The Journal: electronic or print, or both?

Rob Siebers, FNZIMLS, Editor
School of Medicine and Health Sciences, University of Otago, Wellington

There has been concern by some members of the NZIMLS regarding the print version of the Journal. They believe that since Journal articles are freely accessible on the Institute's web site the printed version is no longer required and that elimination of the printed version would help the sustainability of forestry worldwide as 40% of the world's forests are now used for paper.

The NZIMLS Journal is printed by Red-i, Auckland. They became the first print company in New Zealand in 2007 to obtain certification to two premier international standards from the Forest Stewardship Council and the Programme for the Endorsement of Forest Certification Schemes Chain of Custody for sustainable renewable forestry. This ensures that paper used for printing the Journal is sourced from forests that are sustainable managed with a chain of custody up to the printed sheet. Paper sourced by Red-i also meets the New Zealand Government Sustainability Procurement Policy. Additionally, Red-i uses vegetable-based inks for printing that are based on renewable raw materials which are non-mineral oil based.

Certainly, if the Journal was only available electronically, the reader can use the computer to read articles of interest. However, reading the print version of the Journal is better on the eye than staring into the light source of a cathode ray tube. Additionally a journal is like a book, always on hand to go back for interesting bits and can be taken to a quit corner for perusal. You can even scribble your own notes in the print version of the Journal.

So, when you sit down in your comfortable chair with the print version of the Journal in one hand and a glass of chardonnay in the other, you can be assured that in the Institute's choice of Red-i to print the Journal we are ensuring the sustainability of renewable forests worldwide. Sustainability as defined by the United Nations is: "The capacity to meet the needs of the present without compromising the ability of future generations to meet their needs".

In this issue

Anthea Povall compared the leukocyte differential of the CellaVision™ DM96 imaging device to the manual differential and differential values of the Sysmex XE-2100 as part of the requirement for the Massey University BMLSc degree. In their article, Anthea and Chris Kendrick report that the CellaVision™ DM96 correctly pre-classified 69% of all samples, with the reclassified data showing strong correlations with the neutrophil, lymphocyte and eosinophil populations using the Sysmex analyser. The authors conclude that the CellaVision™ DM96 is particularly suited to laboratories processing large numbers of normal samples, but that microscopy and the experienced morphologist continues to occupy an important place in the haematology laboratory.

In their article Parsian and colleagues determined the serum laminin level cutoff point for predicting liver fibrosis. They determined laminin concentrations in chronic hepatitis patients by ELISA and compared them to healthy controls. Serum laminin concentrations were significantly higher in the patient group and determined a cutoff for the various stages of liver fibrosis that showed a good sensitivity and specificity. After treatment laminin levels decreased but were still higher than the control group. The authors conclude that serum laminin is a useful marker of liver fibrosis.

In this issue are two obituaries of prominent members of our profession. Barry Edwards served for 18 years on the NZIMLS Council, 15 years as Secretary. On New Year's Day 2009 an "after match function" was held in memory of Barry, a report of which is elsewhere in the Journal. Robert (Bob) Allan was for many years charge technologist in the biochemistry department of Dunedin Hospital. He previously was Editor of the Institute's Journal for ten years. Both Barry and Bob were passionate about the profession and devoted a great deal of their time to the profession and its members.

Another Journal questionnaire appears in this issue giving members the opportunity to earn 5 CPD points. Since its inception, the Journal questionnaire has proven a popular avenue to accrue CPD points with over 500 members submitting their answers each issue. Although the majority experience no problems submitting through the NZIMLS web site, a few do. One such problem that has been identified is that some employers 'time out' from the web site after a specified time. We suggest that members first write their answers in a word doc and then cut & paste their answers on the NZIMLS web site. If your submission was successful then you will receive your submitted answers in a confirmatory email to the address you have supplied. Because of the shear volume of responses the Editor tries to mark and respond within one to two days of submission. It must be remembered that the Editor is not a paid employee of the NZIMLS and does this work voluntarily. He is allowed to go on annual leave and thus was disappointed to receive a fair number of emails upon his return in February querying why the member had not received an immediate reply to his/her submission.

Annually the NZIMLS awards a prize for the best case study published in the Journal during the calendar year. Joint winners for 2008 were Sarla Naran and Sharda Lallu from Anatomic Pathology at Wellington Hospital for their case study on pulmonary mucormycosis. Congratulations to both. As Editor I look forward to receiving submissions of case studies for 2009. Many excellent case studies are presented at SIG meetings and the North and South Island Seminars. Expect the Editor to attend some of those meetings and tap you on the shoulder to submit to the Journal. Your case study presentation deserves a wider presentation that at the meeting. You will also obtain CPD points and be eligible (if you are a financial member of the Institute) for either (or both) the MedBio Prize for the best paper in each issue of the Journal and the NZIMLS Journal Prize. Indeed, the winners of the NZIMLS Journal Prize for 2008 were also the winners of the MedBio prize for the August 2008 issue.

Rob Siebers
Editor

Estimated platelet and differential leucocyte counts by microscopy, Sysmex XE-2100 and CellaVision™ DM96

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Abstract
Modern imaging techniques and specialised computer software has enabled the differential counting of leucocytes to become automated. This study compared the leucocyte differential of the CellaVision™ DM96 to the manual differential and the differential values from the Sysmex XE-2100. The evaluation was performed on 50 samples submitted for routine CBC testing at Canterbury Health Laboratories in Christchurch. The DM96 correctly pre-classified 69% of all samples, with the reclassified data showing strong correlation with the neutrophil, lymphocyte and eosinophil populations using the Sysmex analyser. The reclassified DM96 results showed a strong correlation with the results obtained by manual differential counting. This study also evaluated the platelet estimation function of the DM96 software and compared the results to manual estimations and the platelet count from the Sysmex analyser. This DM96 method produced a consistent over estimate of platelet numbers and was more time consuming than microscopy. Using a correction to the DM96 multiplication factor, results closer to the actual platelet were obtained.

Key words: leucocyte differential; platelet count; CellaVision DM96; Sysmex XE-2100

N Z J Med Lab Sci 2009; 63 (1): 3-10

Introduction
The increased workload experienced by many haematology laboratories coupled with reductions in staff numbers has lead to the introduction of automated morphological analysis in laboratories. The improved processing capacity of computers combined with high resolution digital imaging technology has allowed for the development of systems which can pre-classify haematological cells, mostly leucocytes (1). The CellaVision™ DM96 is an imaging device intoduced into Canterbury Health Laboratories (CHL) in 2007. This paper reports on the performance of the DM96 to determine the leucocyte differential and compared the results with both manual differentials and the differential result from the Sysmex XE-2100 automated cell counter. In addition, the DM96 derived platelet estimate was performed and compared to the standard microscopic platelet estimate and the platelet count from the Sysmex XE-2100.

Materials and methods
Equipment
The study utilised the Sysmex XE-2100 haematology analyser and the Sysmex SP1000i slidemaker/autostainer. The CellaVision DM96 system was used for the automated leucocyte differential counting and platelet count estimates. These analysers are used in the core laboratory at CHL and provide CBC testing of samples submitted from inpatients and outpatients of the public hospital in Christchurch. Standard light microscopes were used for microscopy.

Sample selection
Samples were selected from the routine work which arrived in the haematology laboratory during a single 24-hour period. Flagging functions on the Sysmex XE-2100 indicated which samples required blood film examination and films were prepared and stained by the Sysmex SP1000i slidemaker/autostainer. Fifty slides were selected for the project by the author's (AP) supervisors from the results of the CBC values. Samples were selected to include both normals and abnormals containing qualitative and/or quantitative abnormalities in the main cell classes. Slides from patients with known acute leukaemia were excluded. The slides were anonymised to show only the laboratory identification numbers. Of the fifty slides four samples were excluded from platelet analysis due to the presence of platelet clumps observed during microscopy. Three samples were also excluded from the differential count analysis as the Sysmex was not able to count suffficient cells to produce a valid differential.

Data collection
Each slide was analysed by the DM96 with 100 cells counted. Cells were pre-classified into: band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils, metamyelocytes, myelocytes, promyelocytes, blast cells, lymphocyte variant forms and plasma cells. For data collection purposes, band and segmented neutrophils were counted together. Lymphocytes, lymphocyte variants and plasma cells were counted as one group. Precursor cells from myeloid blast cells through to metamyelocytes were counted together as immature granulocytes. Unidentified cells were categorised as 'other'. Results were stored as the pre-classification DM96 data for each slide and were then viewed. Incorrectly classified cells were moved to their correct classification based on their morphological appearance. Cells classified as 'other' were similarly reclassified where possible and moved into one of the leucocyte classification groups. Non-leucocyte classifications were also examined and the cells incorrectly identified as nucleated red cells, giant platelets, platelet aggregates, smudge cells or artefacts were reclassified. Results were recorded as the reclassified DM96 data.

For each slide a manual 100-cell differential was performed using a light microscope at 500x magnification. Cells were recorded in the same categories as for the reclassified DM96 data.

The platelet count estimation facility on the DM96 was used to derive an estimate of platelet numbers on each of the stained blood films. Platelet numbers present in 16 sub-images were counted, and the average value for those fields multiplied by the platelet estimation factor (pre-set at 10) provided the estimated platelet result. For the DM96, the platelet estimation corresponded to counting the equivalent of eight high power microscopy fields as per the CellaVision Users Manual (4). Results were recorded as the DM96 platelet estimation.

Manual platelet estimates were performed by counting the

Table 1. Platelet count raw data (x 10 9/L) for slides 1-50.

Slide No	Sysmex	Platelets DM96	Manual	Slide No	Sysmex	Platelets DM96	Manual
1	358	450	354	24	362	539	413
2	173	264	246	25	338	420	419
3	171	151	147	26	255	303	260
4	254	194	233	27	889	1105	974
5	59	66	64	28	190	264	200
6	38	51	30	29	236	280	211
7	165	171	180	30	165	203	221
8	197	259	207	31	232	295	247
9	594	663	545	32	495	483	504
10	142	150	152	33	159	158	158
11	291	345	319	34	514	624	594
12	241	229	201	35	341	510	423
13	128	186	174	36	32	43	40
14	225	283	295	37	175	234	173
15	222	355	244	38	449	556	602
16	222	288	250	39	333	388	355
17	357	496	424	40	445	484	437
18	229	324	275	41	251	225	262
19	480	493	441	42	345	305	285
20	197	270	222	43	354	504	481
21	158	208	231	44	350	326	346
22	371	543	490	45	105	79	96
23	367	449	382	46	360	391	424

* Four samples were clotted and were excluded from all platelet analyses.

Table 2. R^2 values for Sysmex vs DM96 and manual differentials for each cell class.

Cell type	DM96		Manual
	Pre-classification	Reclassified	
Neutrophils	0.91	0.92	0.93
Lymphocytes	0.85	0.84	0.77
Monocytes	0.70	0.17	0.45
Eosinophils	0.69	0.71	0.81
Basophils	0.12	0.12	0.24
Immature grans	0.44	0.48	0.05

number of platelets in 10 successive high power fields at 1000x magnification and multiplying the count by 109 to give the platelet estimation as n/10^9/L. These results were recorded as the manual platelet estimation.

Following collation of manual and DM96 results for both the leucocyte differentials and the platelet estimates, patient data from the laboratory information system was accessed and the verified data for the Sysmex differential and platelet count were obtained. Correlation data was derived using Bland-Altman plots and regression analysis.

Results

Platelets

The platelet estimates from the DM96 and the visual method followed closely the platelet counts generated by the Sysmex XE-2100 (Table 1 and Figure 1). Of interest was the tendency for the DM96 to over estimate the platelet count as compared with the manual method. In comparison with the Sysmex results, the R^2 values were 0.91 and 0.93 for the DM96 and visual platelet estimation methods, respectively (Figure 2).

The average ratio of Sysmex to DM96 platelet counts was 0.86 with the DM96 counts an overestimate in 80% of samples. In absolute values the average platelet overestimate was about 66 platelets. The average ratio of Sysmex to visual platelet counts was 0.93 with the visual result either over- or underestimating the Sysmex result by about 40 platelets.

Leucocyte Differential

The correlation between the Sysmex data and either reclassified DM96 or visual results for cell lines are summarised by the R^2 values in Table 2. The correlation data presented for the leucocyte counts in Table 2 was derived from the data presented in figures 3-14.

Both DM96 and manual methods showed a strong correlation with the Sysmex data for the neutrophil counts, and an acceptable correlation was observed for lymphocyte and eosinophil counts. The correlations for monocytes, basophils and immature granulocytes were weak. Table 2 also shows the R^2 values for the pre-classification data. The pre-classification data was correct in 69% of cases. In most cases the results paralleled the reclassified results, except for the monocyte population, where the pre-classification count correlated strongly with the Sysmex data.

The percentage agreement with Sysmex data for each cell class according to test method is presented in Figure 15. The results from each method were divided into the proportion of test results which were in agreement with the Sysmex data, and those results which were not. Results which were not in agreement were further divided according to whether the result was an over- or underestimate of the Sysmex value.

All test methods identified neutrophils with the highest percentage agreement, followed by lymphocytes. Cell classes with smaller populations represented in the blood generally showed a lower percentage agreement.

Figure 16 shows the proportion of each cell class in an average differential for each test method. On average, all test methods had higher neutrophil, and lower lymphocyte and monocyte counts compared to the Sysmex. All methods were approximately equal in their estimates of smaller cell classes. Overall, values obtained by the DM96 and manual methods were almost identical.

Discussion

Both DM96 and visual platelet estimates correlated well with the Sysmex XE-2100 platelet counts, however, there was a consistent overestimation of the DM96 platelet count. The overestimation could be accounted for by the platelet estimation factor used on the machine, which set at 10 appeared to be too high. A change of the estimation factor to 0.86 would yield a platelet estimation very close to the Sysmex platelet count making this method more accurate than the manual method. Forty-seven samples were analysed in this study which was more than the 30 sample minimum recommended by the manufacturer in the setup of the platelet estimate function (3). The slides used in the study included 3 samples with platelet counts below 100 x 10^9/L and 5 above 450 x 10^9/L. Given these small numbers it may be worthwhile repeating the estimated platelet count on larger numbers of samples containing platelet numbers above and below the normal range.

While both the platelet estimate methods were time consuming, the DM96 method took longer to scan slides to estimate the platelet numbers in the film. An advantage of the DM96 method was the overlay grid which aided counting, but the major disadvantage was poor image quality. In our hands the imaging was sometimes out of focus. With traditional microscopy a scratched section could be bypassed, but the DM96 has no 'reserve' images for such a situation.

While the platelet estimate function of the DM96 could at times be useful in the laboratory, platelet estimates are rarely required. The DM96 is capable of producing accurate platelet estimates once properly setup, but its use for screening all slides probably cannot be justified given the extra time taken.

In this study the identification and counting of neutrophils and lymphocytes by manual and DM96 methods showed good correlation with the Sysmex data although the results as mentioned showed both over- and under-estimation by both techniques. The smaller cell populations (eosinophils, basophils and blast cells) showed less variation in the results between the the manual and DM96 methods, however, these did not correlate well with the Sysmex data, the eosinophil population being the exception. The DM96 to Sysmex correlation was particularly poor for the monocyte population, and immature granulocytes were not well identified by the manual method when compared to the Sysmex values.

The pre-classification data correlated well with the reclassification values in most cases except for the monocyte population, which had a high pre-classification R^2 value. This resulted from other cells (often smudge cells) being incorrectly classified as monocytes.

In a previous study by Briggs et al the pre-classification monocyte count as compared to the manual method resulted in a low correlation (2). Monocytes are generally poorly classified by both automated and manual differential systems, mainly due to smear method and the area of the film examined (4). The relatively low number of monocytes in most samples is also likely to contribute to this error.

Sample size is an important factor to consider when assessing the relevance of results. In this study 50 samples were initially selected, of which 46 were used for the platelet estimates and 47 for leucocyte analyses. Three other studies compared the DM96 and manual differential, analysing 136, 322 and 400 samples respectively, and performed either 200 or 400-cell differentials (2,4,5). The manual 100 cell differential is the standard method used in laboratories today. This method lacks both accuracy and precision which can be improved on by counting 200-400 cells. This was not performed in this study. Additionally, the comparison of the manual differential results to the Sysmex XE-2100 differential, which counts many thousands of cells, highlighted the manual differential inaccuracies. The DM96 and manual methods counted the same number of cells and neither had any statistical advantage over the other.

One recurrent source of error was the total number of cells present in the reclassification differential. As only 100 cells were counted and classified by the DM96 in our study, when reclassification took place the differential was more or less than 100 cells in

Figure 1. Comparison of the Sysmex platelet count and the estimated DM96 and manual platelet count.

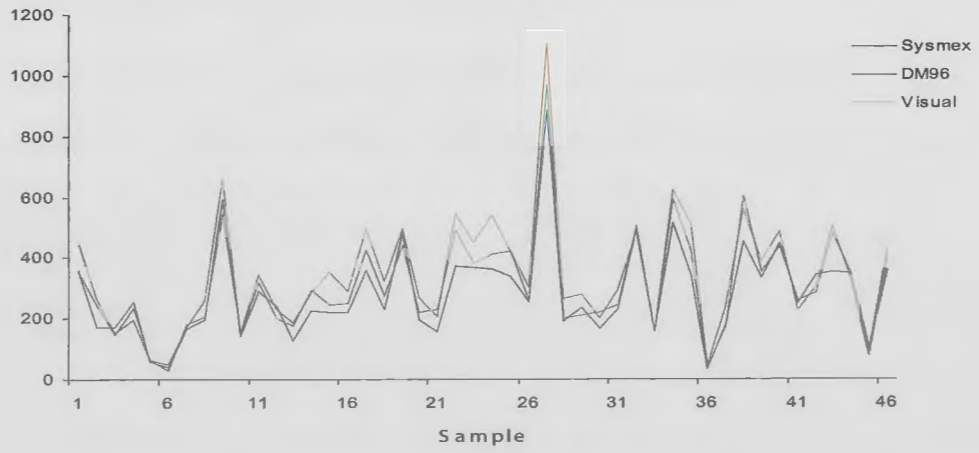


Figure 2. Correlation between the Sysmex platelett counts and either DM96 or visual platelet estimates.

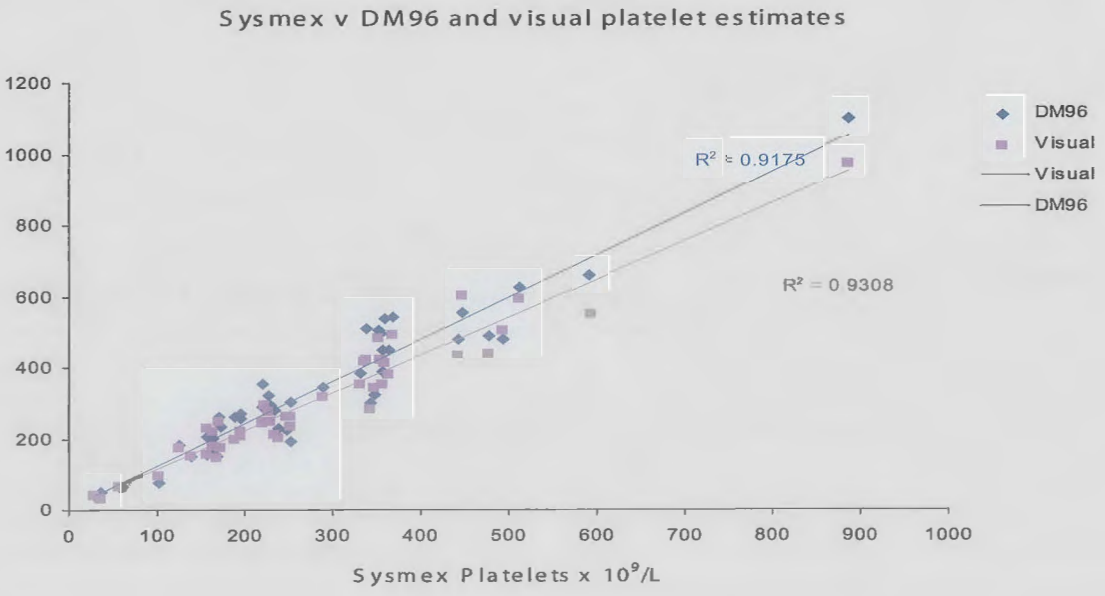


Figure 3. Sysmex v DM96 neutrophil count

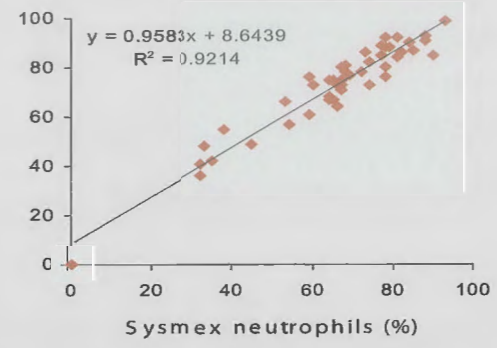


Figure 4. Sysmex v manual neutrophil count

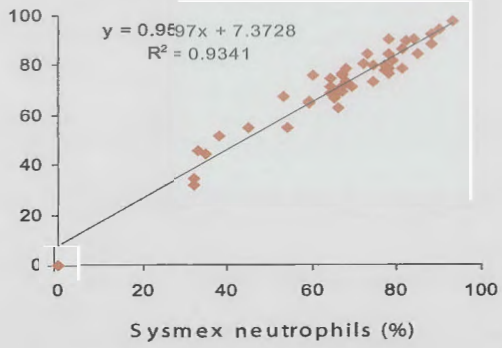


Figure 5. Sysmex v DM96 eosinophil count

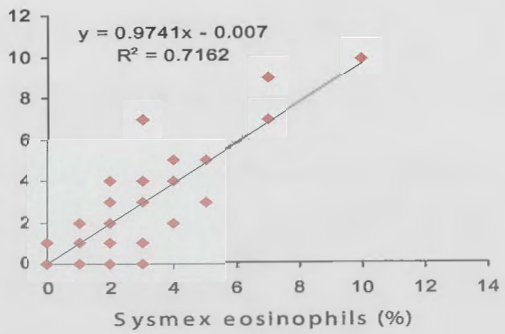


Figure 6. Sysmex vs manual eosinophil count

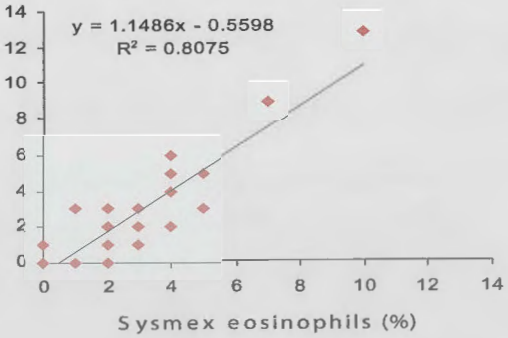


Figure 7. Sysmex v DM96 lymphocyte count

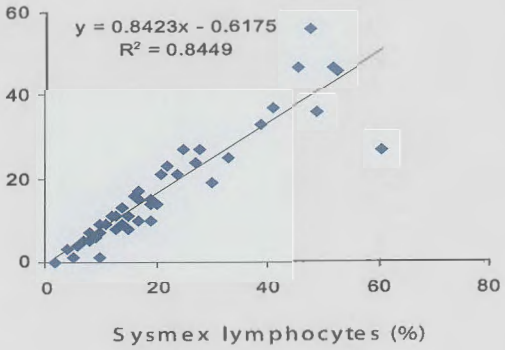


Figure 8. Sysmex v manual lymphocyte count

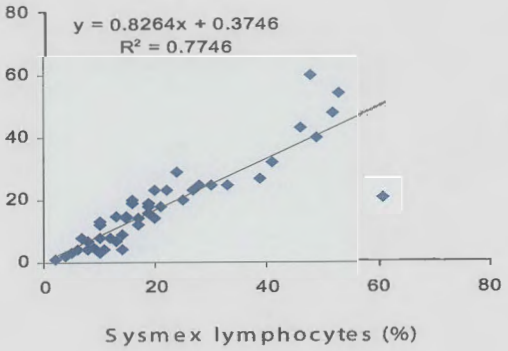


Figure 9. Sysmex v DM96 basophil count

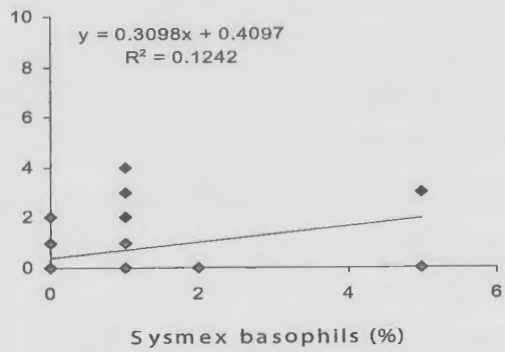


Figure 10. Sysmex v manual basophil count

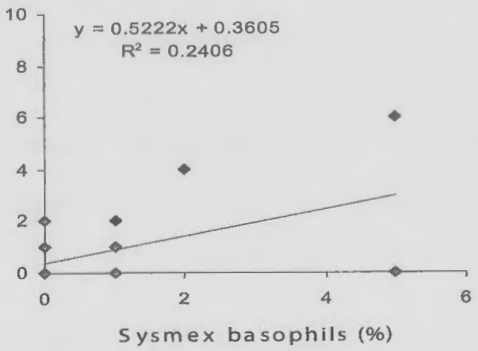


Figure 11. Sysmex v DM96 monocyte count

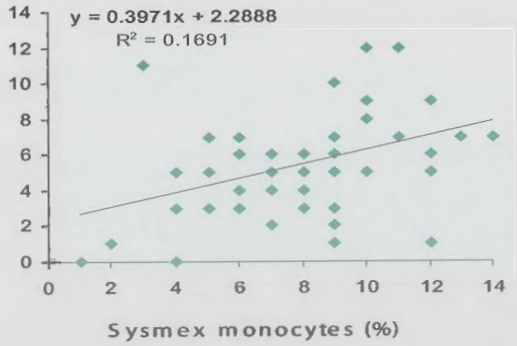


Figure 12. Sysmex v manual monocyte count

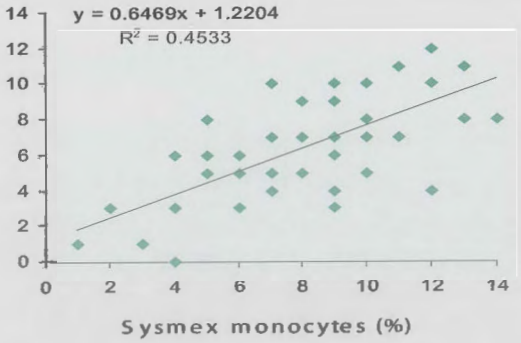


Figure 13. Sysmex v DM96 immature granulocyte count

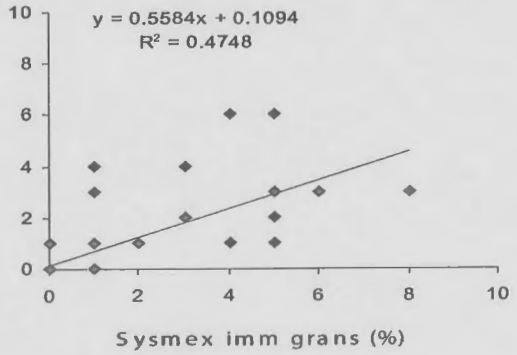


Figure 14. Sysmex v manual imm granulocyte count

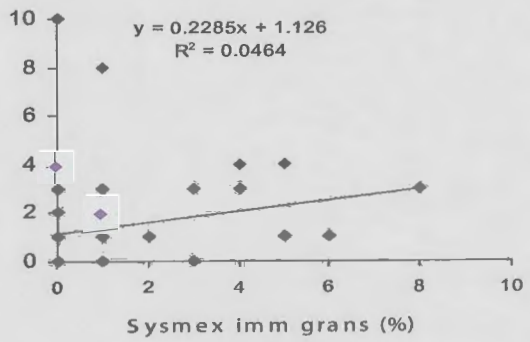
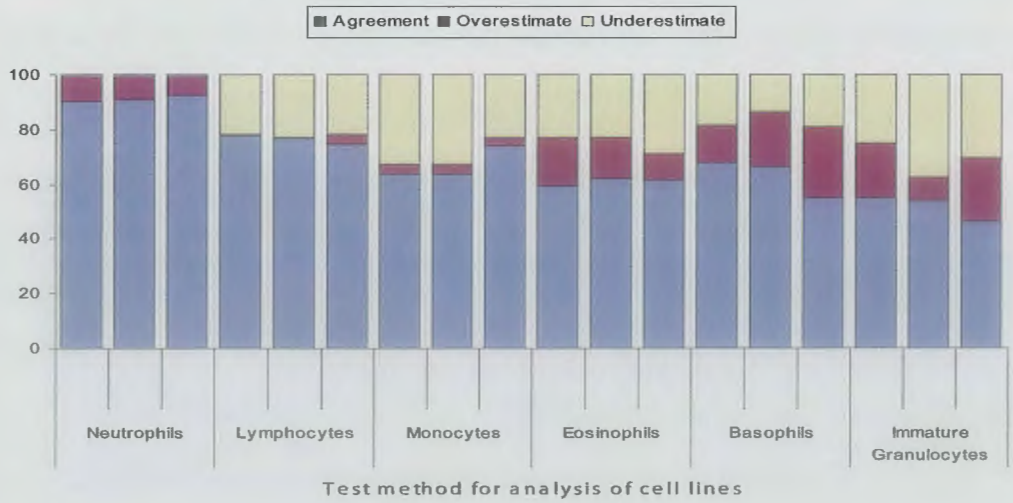
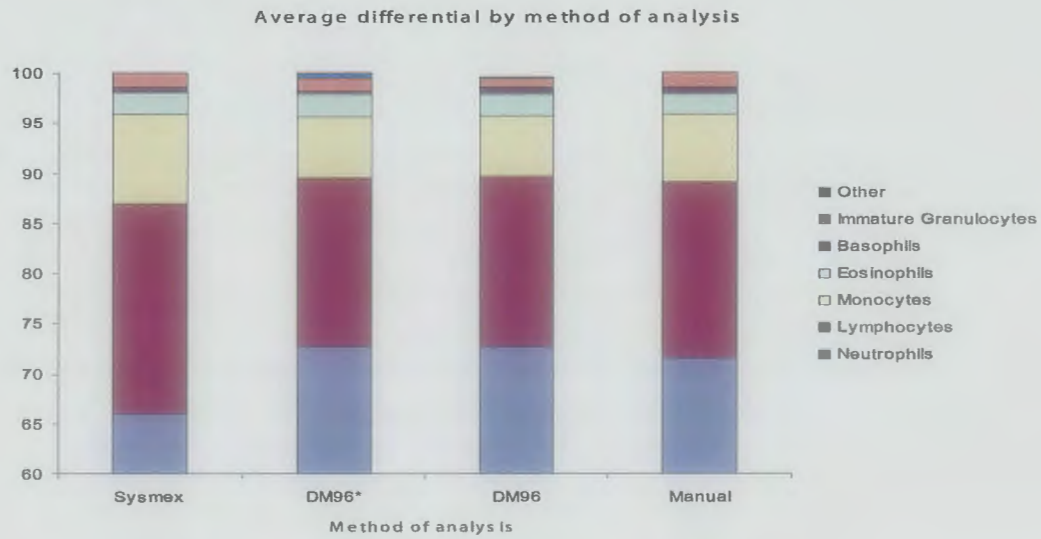


Figure 15. The percentage agreement with Sysmex data for each cell class according to test method.



* Pre-classification data.

Figure 16. The proportion of each cell type in an averaged differential for each test method.



* Pre-classification data.

half of the samples. Where a 'reciprocal reclassification' took place (eg. a neutrophil classified as an eosinophil as well as an eosinophil classified as a neutrophil), the differential remained 100. However, when cells were classified as non-leucocytes or vice versa, the differential was higher or lower than 100 cells after the reclassification. Other studies have set the automated microscope to count 105, 110 or 200 cells to take this problem into account (2,4,5). In our experience setting the count value to 110 should be adequate for most samples.

It is also worth remembering that the DM96 only examines the area of the slide where it can detect an erythrocyte monolayer, thus if a sample does not contain many leucocytes in that area, the differential value may be provided on less than 100 WBCs. In this respect traditional microscopy has the advantage in that an area with overlapping erythrocytes can still be examined and leucocytes accurately identified.

Another important factor to take into account when examining correlation data, is the experience of the morphologists examining the blood films. Logically it would be expected that more experience would correlate with greater accuracy of identification, and this has been shown to be the case in other studies (2).

In conclusion, this study has shown that the results from a 6-part differential (including immature granulocytes) performed by the DM96 is similar to the results obtained by a manual differential. Neutrophil, lymphocyte and eosinophil values can be expected to correlate well with results obtained from the analyser differential using the Sysmex XE-2100, however monocyte, basophil and immature granulocyte numbers can be expected to correlate less well. This work supports the findings of others that the DM96 is particularly suited to laboratories (eg. community laboratories) processing large numbers of normal samples (2). More complex samples from patients with haematological malignancies, recent bone marrow transplantation and morphological changes associated with some infections, require the attention of a morphologist to accurately identify and classify immature cells. In the setting of a larger laboratory, such as that at Canterbury Health Laboratories, the DM96 can reduce the number of 'normal' films examined by morphologists, and can be used in conjunction with microscopy to investigate abnormal cases. The results of this study have demonstrated that microscopy and the trained morphologist still occupy an important place in the haematology laboratory. For the moment at least the human eye continues to occupy the high ground ahead of the modern mechanical morphologist.

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Attenuation of serum laminin concentrations upon treatment of chronic hepatitis

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Abstract

Objectives: The aim of this work was to determine the serum laminin level cutoff point for predicting liver fibrosis highlighting its diagnostic value and determining the effect of treatment on serum laminin concentrations.

Methods: Serum laminin concentrations in chronic hepatitis patients (n=62) and controls (n=20) were compared by ELISA and stages of fibrosis were assessed according to the modified Knodell score system.

Results: Mean serum laminin concentration in patients (91.9 ± 20.9 ng/ml) was greater than controls (46.2 ± 10.2 ng/ml; p <0.001). Serum concentrations of laminin in all stages of hepatic fibrosis were significantly higher than those of healthy controls (p <0.05). A cutoff point of 52ng laminin/ml of serum was obtained for the discrimination of various stages of liver fibrosis showing a good sensitivity (96.8%) and specificity (80%). After 6 months of treatment, a gradual decrease in serum laminin concentrations were observed, however the level was still higher than that of the healthy group (p<0.05).

Conclusions: Our findings suggest that the serum laminin concentration is a useful noninvasive marker of liver fibrosis and shows a strong positive correlation with different stages of the disease.

Key words: chronic hepatitis; hepatic fibrosis; laminin; treatment

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Introduction

Laminin was initially identified by Timpl and Martin in 1979, from a murine fibrosarcoma (1). Laminin is one of the main glycoproteins of the basement membrane and participates in a series of such biological phenomena such as adhesion, migration, cellular differentiation and the maintenance of the cytoskeleton upon its binding to several components of the matrix, such as collagen type IV, heparin sulphate and entacin (2-7).

In the liver, laminin is normally found around the vessels and biliary ducts, where basement membranes are identified. Little or only a slight reaction for antibodies against laminin can be observed in the hepatic sinusoids (8, 9). In this organ, glycoproteins are also involved in intracellular activities, such as the normal differentiation of the biliary ducts, expression of albumin messenger RNA in hepatocytes, and regeneration with normal lobular organization following partial hepatectomy (10-12). Laminin is thought to be synthesized by hepatocytes and sinusoidal cells (13). Among all cellular types in the sinusoids, special attention should be given to stellate cells or lipocytes, which produce the largest amount of serum laminin. With the development of hepatic cirrhosis, laminin and collagen deposition occurs both along the fibers of septal fibrosis and subendothelial sinusoids or Disse's space. At the latter site, laminin deposition, together with collagen deposition, determine the formation of a true basement membrane along sinusoids. This phenomenon is called capillarization of Disse's space (14).

Increased concentrations of laminin were observed in the more advanced stages of fibrosis in patients with hepatic disease (15-19). Kropf et al have proposed laminin serum concentrations as a sensitive screening test for hepatic fibrotic disease and portal hypertension (18, 19).

An important component of the management of hepatic fibrosis is the clinical assessment of disease severity. Liver histology is frequently considered the gold standard for establishing the severity of hepatic necro inflammation and fibrosis. However, liver biopsy is an invasive procedure that may cause undesirable events, such as pain in 20% to 30% of cases, major complications in 0.5%, and even death. In addition, because of the complications derived from the procedure and frequent poor patient acceptance, the direct costs of such procedures are high (20). Thus, the finding of surrogate markers of liver fibrosis could be relevant to reduce the number of liver biopsies in patients with hepatitis.

The first aim of this study was to determine the serum laminin level cut off point to predict both presence and absence of fibrosis. The second aim was to obtain a relationship between the diagnostic values of serum laminin concentrations for differentiation of various stages of hepatic fibrosis in patients with chronic hepatitis. The final aim was to determine serum laminin changes during treatment of these patients.

Materials and methods

Study population

62 patients (35 men and 27 women, mean age ± SD: 35.4 ± 11.3; range: 15-65 years) were enrolled in the study. Among these, 35 patients had hepatitis B virus (HBV), 14 had hepatitis C virus (HCV), and 13 were autoimmune hepatitis (AIH). The subjects were selected from persons who were referred to the Gastroenterology Research Centres in Tabriz and Gonbad, Iran. Patients were included in the study if they were positive for serum hepatitis B surface antigen or C antibodies and had persistently elevated serum aminotransferase concentrations greater than 1.5 times the upper limit of the reference range for at least six months. All patients were diagnosed according to the International Autoimmune Hepatitis Group Report protocol (21).

For assessment of liver fibrosis scores all patients underwent liver biopsy as part of the normal diagnostic procedure and were sub-classified according to the score for the histological activity index (HAI). Patients with a history of gastrointestinal bleeding and chronic liver disease (Wilson's disease, hemochromatosis, a1pha 1-antitrypsin deficiency, biliary disease, hepatocellular carcinoma), active intravenous drug abuse, and liver transplantation were excluded.

Control sera for the determination of laminin were obtained from 20 healthy individuals; 10 women and 10 men, 20-69 years old (mean ± SD: 42 ± 14.7 years).These healthy persons had normal serum concentrations of aminotransferases and alkaline phosphatase (ALP) and had no history of gastrointestinal bleeding or chronic liver disease, smoking, alcohol intake, no family history of hepatitis or liver disease, and no active intravenous drug abuse, or liver transplantation. All patients gave written informed consent to use these data for scientific purposes and the study was approved by Tabriz University of Medical Sciences Ethical Committee.

Blood sample collection and analysis

Fasting venous blood (5ml) was collected on the day before the beginning of the treatment and three times at two monthly intervals, i.e. two, four and six months after the beginning of treatment. Serum was separated (2500 g for 5 minutes) within one hour of blood collection. Standard liver function tests (LFT), including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin, albumin (Alb), and hepatitis serology were performed on aliquots of each sample at entry and recorded. The rest of the serum samples were stored at – 20°C. Serum laminin concentrations were determined in one analytical batch. The controls were dealt with in the same manner, except that the control group provided blood only once at entry. Routine LFT were performed using commercially available kits (Ziestchem, Iran).

Patients treatments were begun if they met the inclusion criteria and they were followed up for at least six months. Treatment of each patient was according to a standard protocol as follows. Hepatitis C patients were treated with Pegylated Interferon + Ribaverin or Interferon + Ribaverin. Hepatitis B patients were treated with Interferon or Adefovir and the AIH patients with Prednisolone and Imuran (22).

Serum laminin concentrations were assayed using a laminin EIA Kit (Takara Bio, code number: MK107) on an ELISA reader (BDSL, Immunoscan, Switzerland, Lab System). The laminin EIA kit is a solid phase EIA based on a sandwich method that utilizes two mouse monoclonal anti–laminin antibodies to detect laminin by a two-step procedure. One of the monoclonal antibodies is bound to the microtitre plate to create the solid phase. Non-specific binding is blocked using a blocking buffer. Samples and standards are then incubated in the microtitre plate wells. After washing the plate, the second anti-laminin monoclonal antibody that is labeled with peroxidase (POD) is added to the wells and incubated. During these steps, laminin is captured onto the solid support on one side and tagged on the other by POD-anti-laminin. The reaction between POD and substrate (H_2O_2 and tetremethybenzidine) results in color development with intensities proportional to the amount of laminin present in the samples and standards. The amount of laminin was determined by measuring the absorbances using an EIA plate reader. A standard curve of 5, 10, 20, 40, 80, 160 and 320 ng/ml laminin was used to convert sample absorbances into ng laminin/ml serum.

Histological assessment of liver damage

All patients underwent a liver biopsy for assessing the presence and severity of liver disease. The biopsy fragments were fixed in a 10% formalin solution for 12 hours and embedded in paraffin. Sections were stained with hematoxylin-eosin, Masson's tri chrome and reticulin stain to establish the histological diagnosis and the extent of the liver lesions.

Specimens were graded and staged according to the modified Knodell scoring system (23, 24). The grading system scores 0-18 and was based on sum of four indices:

1. Periportal or periseptal interface hepatitis (piecemeal necrosis, score 0-4)
2. Confluent necrosis (score 0-6)
3. Focal (spotty) lytic necrosis, apoptosis, and focal inflammation (score 0-4)
3. Portal inflammation (score 0-4)

The fibrosis scores were determined as Stage 0 if there was no fibrosis, Stage 1 if there was fibrous expansion of some portal areas, with or without short fibrous septa, Stage 2 if there was fibrous expansion of most portal areas, with or without short fibrous septa, Stage 3 if there was fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging, Stage 4 if there was fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)], Stage 5 if there was marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis), and Stage 6 if there was probable or definite cirrhosis (23,24).

Statistical analysis

All statistical analyses were done by SPSS version 12.0 for Microsoft Windows (SPSS Inc.) and the data were considered statistically significant at a two-sided $p < 0.05$. Numerical data were expressed as mean ± SD. According to the Gaussian distribution (1 sample Kolmogorov-Smirnov test), mean of serum laminin concentrations of patients and various chronic hepatitis stages, as well as the control group, were compared using the Mann-Whitney U-test or Student's t test. Spearman's correlation coefficients were calculated to assess the relationship between the histological degree of severe liver fibrosis and the concentrations of serum laminin.

To assess and compare the diagnostic accuracy of laminin for differentiating chronic hepatitis patients with severe liver fibrosis from those without fibrosis, we plotted ROC curves (25) and calculated the areas under the curves (AUC) for comparison. Receiver operating characteristic (ROC) curves were generated by plotting the relationship of the true positivity (sensitivity) and the false positivity (1 - specificity) at various cutoff points of the test. An AUC of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value (26). The diagnostic sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) values were also calculated.

Results

Serological and biochemical profiles of the patients are summarized in Table 1. Hepatitis serology revealed that 56.4% of the patients were suffering from chronic hepatitis B, 22.5% from chronic hepatitis C and 20.9% from autoimmune hepatitis. Histological examination of liver for fibrosis scoring revealed 35% of patients to be suffering from significant fibrosis (stage ≥3).

The mean serum laminin concentrations (ng/ml ± SD) in patients

Table 1. Serological and biochemical profile of patients and control group.

Variables	Patients (n=62)	Control group (n=20)
Male/Female (No)	35 / 27	10 / 10
Age in years (mean ± SD)	35.4 ± 11.3	42 ± 14.7
ALT (mean ± SD) Reference range: < 38 IU/L	132.7 ± 141.7	27.3 ± 6.4
AST (mean ± SD) Reference range: < 42 IU/L	97.0 ± 138.3	28.3 ± 6.5
ALP (mean ± SD) Reference range: women 64-305 IU/L; men 80-306 IU/L	376.4 ± 413.7	130.6 ± 38
Total bilirubin (mean ± SD) Reference range: <20.5 µmol/L	32.5 ± 58.1	-
Direct bilirubin Direct (mean ± SD) Reference range: < 6.8 µmol/L	11.5 ± 23.1	-
Albumin (mean ± SD) Reference range: 35.0-52.0 g/L	41.0 ± 6.0	-
Hemoglobin (mean ± SD) Reference range: women 12.0-16.0 g/L; men 14.0-18.0 g/L	13.1 ± 2.6	-
Hematocrit (mean ± SD) Reference range: women 37.0-47.0%; men 40.0-54.0%	41 ± 9.1	-
Platelets (mean ± SD) Reference range: 160,000-450,000	220,511 ± 132,732	-
Smoking (No, %)	7 (11%)	0 (0%)
Alcohol intake (No, %)	1 (1.6%)	0 (0%)
Family history of hepatitis (No, %)	5 (8%)	0 (0%)
Family history of chronic liver disease (No, %)	1 (1.6%)	0 (0%)
History of drug abuse (No, %)	2 (3.2%)	0 (0%)
Chronic hepatitis B (No, %)	35 (56.4%)	0 (0%)
Chronic hepatitis C (No, %)	14 (22.5%)	0 (0%)
Autoimmune hepatitis (No, %)	13 (20.9%)	0 (0%)
Fibrosis Stage 0 (No, %)	10 (16.1%)	0 (0%)
Fibrosis Stage 1 (No, %)	19 (30.6%)	0 (0%)
Fibrosis Stage 2 (No, %)	11, 17.7%	0, 0%
Fibrosis Stage 3 (No, %)	9, 14.5%	0, 0%
Fibrosis Stage 4 (No, %)	8, 12.9%	0, 0%
Fibrosis Stage 5 (No, %)	4, 6.4%	0, 0%
Fibrosis Stage 6 (No, %)	1, 1.6%	0, 0%

Table 2. Comparison of serum laminin concentrations (ng/ml, mean ± SD) of patients in various stages of liver fibrosis and various stages of sampling vs. healthy controls.

Laminin concentrations	Fibrosis Stage 0	Fibrosis Stage 1	Fibrosis Stage 2	Fibrosis Stage 3	Fibrosis Stage 4	Fibrosis Stage 5
At entry	63.0 ± 12.1†	85.7 ± 6.3‡	94.0 ± 10.2‡	100.6 ± 8.6‡	104.2 ± 20.2‡	130.2 ± 13.7‡
2nd month	55.4 ± 8.5†	79.5 ± 6.6‡	90.9 ± 12.0‡	96.0 ± 7.0‡	100.4 ± 18.4‡	118.2 ± 19.0‡
4th month	49.2 ± 6.0*	75.3 ± 7.0‡	85.7 ± 12.4‡	90.0 ± 5.0‡	93.0 ± 15.2‡	111.7 ± 12.6‡
6th month	46.2 ± 4.5*	75.2 ± 6.0‡	83.8 ± 11.1‡	87.6 ± 5.9‡	88.9 ± 13.0‡	110.2 ± 9.5‡

Results are mean ± SD. Differences: *statistically not significant; †p <0.05; ‡ p <0.001

with HBV, HCV and AIH were 92.0 ± 20.9 (range: 63-149), 92.8 ± 24.2 (range: 43-128), 90.6 ± 18.4 (range: 69-128), respectively. However, the mean serum laminin concentrations (ng/ml ± SD) in healthy control subjects were statistically significantly lower than the patients serum laminin concentrations (46.2 ± 10.2; $p < 0.001$, Figure 1).

In Table 2, mean ± SD of serum laminin concentrations in various chronic hepatitis stages and various stages of sampling are presented. As shown in this table, differences in serum concentrations of laminin, almost in all stages of hepatic fibrosis as compared with the healthy controls, were not statistically significant ($p < 0.05$). An exception was in 3rd and 4th samples of patients in Stage 0 ($p=0.313$ and 0.985, respectively). Also in fibrosis Stage 6, which was represented by only one patient, we could not compare this single patient with the control group (laminin serum concentration in this stage: 143 ng/ml). As the degree of liver fibrosis stage increased, there was a gradual rise in basal serum laminin concentrations at entry ($rS=0.788$, p-value<0.001, Figure 2).

After the beginning of treatment, a decrease in serum laminin concentrations was observed. We compared serum laminin concentrations of patients after six months of treatment with the basal laminin concentrations (at entry) of each fibrosis stage. Although there was a gradual decrease in serum laminin concentrations of progressive fibrosis stages (i.e. Stages 4 and 5), differences were not statistically significant ($p < 0.093$ and < 0.054, respectively). Conversely in early stages of fibrosis (i.e. Stages 0-3) differences between the serum laminin concentrations at entry compared with the laminin concentrations after the beginning of treatment were statistically significant (for Stages 0 and 1, $p < 0.001$; and for Stages 2 and 3 , $p < 0.05$).

Table 3 shows the cutoff point, sensitivity, and specificity of serum laminin concentrations. In this table the ROC curve data, for patients before and after the treatment, are presented. Figure 4 illustrates the ROC curve of serum laminin concentrations in patients (at entry) for differentiation of patients with liver fibrosis vs. control group.

Discussion

Several serum markers have been developed to assess fibrogenesis and investigations have been made to replace liver biopsy with non-invasive markers of liver fibrosis, which have been assessed in many studies. However, questions remain regarding their sensitivity and significance and whether changes in concentrations of these markers during the treatment protocol can be detected (27). In our study we have assessed serum laminin concentrations in patients with chronic hepatitis in an attempt to evaluate its predictive value for the risk of fibrosis progression. In addition, we have investigated changes in serum laminin concentrations during the treatment protocol.

As shown in Table 2, the mean serum laminin concentrations in patients with chronic liver disease were significantly higher than those of healthy controls. These results are consistent with other reported studies (16, 28). As the stage of liver fibrosis increases, there is a rise in serum laminin concentrations (Table 2) and patients in a higher fibrosis stage show higher serum laminin concentrations. In addition, as is clear from Figure 2, the correlation of serum laminin concentrations with various histological stages of liver fibrosis revealed a strong positive correlation ($rS= 0.788$). This indicates a positive relationship between serum laminin concentrations and the degree of liver fibrosis, and the two variables are linearly related ($p < 0.001$). As shown in Table 2, differences in serum concentrations of laminin at various stages during the treatment protocol, compared to healthy controls, were statistically significant ($p < 0.05$). It seems that during the treatment protocol there was a decrease in serum laminin concentrations. After six months of treatment, gradual decreases in serum laminin concentrations were observed. When we compared basal serum laminin concentrations in patients (at entry) with the concentrations of laminin after 6 months of treatment, we observed that the serum laminin concentrations did not differ statistically in stages 4 and 5 of liver fibrosis. Conversely, in the early stages of liver fibrosis (0-3) the differences were statistically significant ($p < 0.05$). Therefore, it appears that treatment is more effective in the early stages of liver fibrosis, because only in patients with a liver fibrosis score of less than 4 a decrease in serum laminin concentrations occurred after treatment. An increase in the inflammation grade of liver damage led to a rise in serum laminin concentrations, and the correlation is statistically significant (data not shown).

As shown in Table 3, a cutoff point of 52 ng laminin/ml serum was obtained for discrimination of various stages of liver fibrosis with a reasonably good sensitivity, specificity, PPV and NPV. The AUC of serum laminin ROC curve was found to be 0.974 indicating that serum laminin is a useful diagnostic index of liver fibrosis. We also used this cutoff point (52 ng/ml) for creating the ROC curve after treatment. Again, the results showed a reasonably good AUC, sensitivity, specificity, PPV and NPV. We conclude that this cutoff point is also suitable for discrimination of our patients with fibrosis during treatment.

Our data are consistent with the work of other groups who reported that serum laminin concentrations increase in chronic liver disease (28-36). Schneider et al (16) and Castera et al (37) reported that laminin concentrations increase in early stages of chronic liver disease and the highest concentrations were in active cirrhosis and chronic active hepatitis.

Several mechanisms are proposed for the elevation of serum laminin concentrations in chronic hepatitis patients. Besides the increased production of laminin in the liver, an additional effect due to a lack of degradation of this protein by liver endothelial cells should also be considered. As demonstrated by Smedsrod et al (38), apart from an increase in tissue deposition or turnover, there would be a decrease in the liver's ability to degrade this protein. In a study of alcoholic liver disease patients, serum laminin P1-peptide concentrations were higher on admission (alcohol intake period) and rapidly returned toward the normal range through alcohol abstinence (39).

In our study we observed that after the beginning of treatment a gradual decrease occurred in serum laminin concentrations. Possibly treatment of liver fibrosis causes the liver endothelial cells to regenerate and new endothelial cells degrade this glycoprotein. Further studies are needed to gain a complete understanding of laminin metabolism.

In conclusion, the findings of our study suggest that serum laminin is a useful non-invasive marker of liver fibrosis. There was a strong positive correlation between serum laminin concentrations and the degree of liver fibrosis and inflammation (all stages and grades). Serum laminin concentrations may also be used for the follow-up of liver fibrosis in patients with chronic liver disease as well as for the assessment of liver fibrosis where liver biopsy is contraindicated.

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Table 3. ROC curve of laminin serum level for discrimination of patients with liver fibrosis vs. control group (laminin cutoff point =52 ng/ml) before and after treatment.

Stages of sampling	AUC (95% CI)	P	Sensitivity	Specificity	PPV	NPV
Before treatment	0.974 (0.945 -1.004)	<0.001	96.8%	80%	93.7%	88.8%
After treatment	0.926 (0.837-0.980)	<0.001	83.9%	80%	92.8%	61.5%

AUC: area under the curve. 95% CI: 95% confidence interval. PPV: positive predictive value. NPV: negative predictive value.

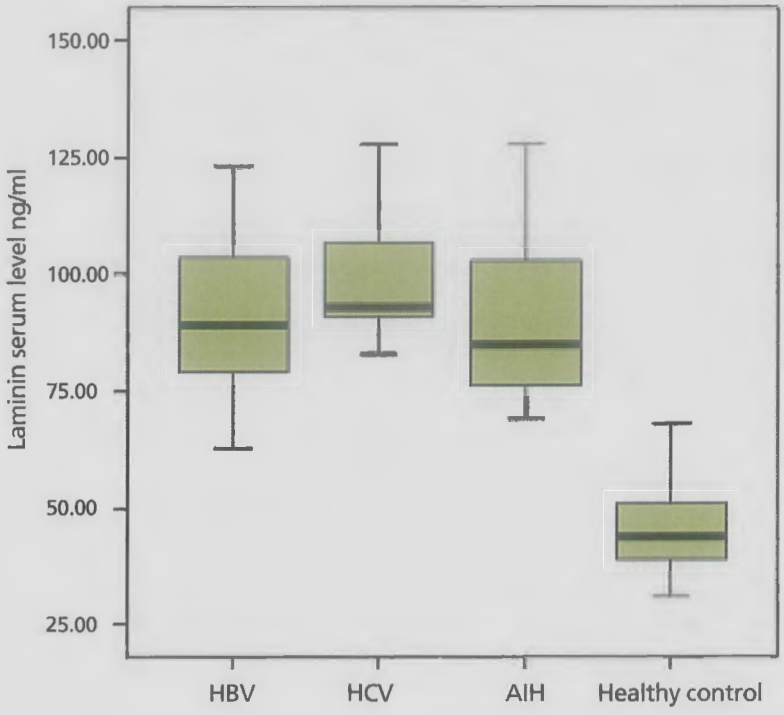

Figure 1. Box plot for serum laminin concentrations (ng/ml) in patients with hepatitis B virus (HBV), hepatitis C virus (HCV), autoimmune hepatitis (AIH) and control groups, at entry.

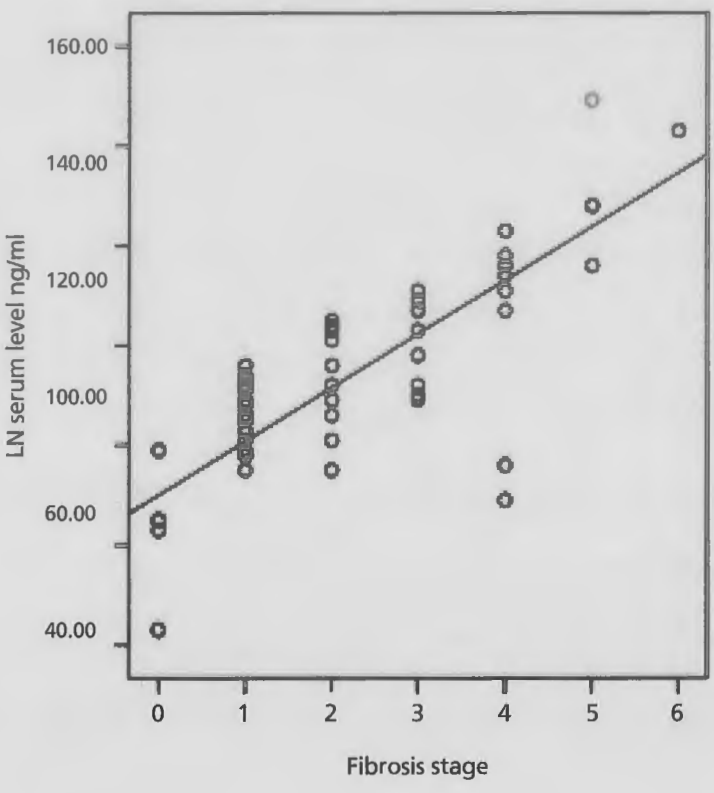

Figure 2. Correlation between serum laminin concentrations (at entry) and stages of fibrosis in liver biopsies ($rS=0.788$, $p < 0.001$).

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Letter to the Editor

Talk about flying blind!!!

In early November 2007 a notice came around calling for volunteers. I love the word 'volunteer' so being the do gooder I am (aka sucker), up my hand went ... for what I did not know but hey, I felt good about myself!! And here the journey starts ....

The Organising Committee started out with 12 members, all ready and rearing to go. Trevor was the man at the helm (or that's what us females let him think), a few old hands and a couple of fresh, youthful, wonderful faces (if you hadn't guessed this is the group I like to include myself in). Meetings were set to occur monthly and so it began with Fran always at the ready with her laptop.

First order of business was a flyer and catch phrase. Next, a scientific Committee for the Educational sessions and workshops.

Again I answered the call for volunteers (by this stage I was feeling absolutely great about myself ... no need to sponsor a child in Africa this year!!) but things were looking up, another fresh face in the form of Cat stepped in to bolster us. Again meetings for the scientific committee were set down to be a monthly occurrence ... I am sure the free lunches had nothing to do with the 100% attendance (the student in us is hard to hide when it comes to seeking out free food) . We were set to go.

Workshop: my history in this was next to nil having being to only one other (there was free food at that too). Title? Speakers? Full day or half day? Costs? Duration of talks? At this point my brain was about to explode and on top of this was the weight of planning the .... Food, I knew most attendees would measure the success or failure of my workshop on how well they ate afterwards (see I do know you all!!). The poor long suffering team at the Dunedin Blood Bank became my sounding post and discussions about the conference were the norm. The Blood Bank teams support and contribution was immense. Hence, my education of being in an Organising committee.....

While it was a busy few months of organizing, stressing and hair discoloring etc etc it was all worth while. Fran kept us all in line. It was fun, rewarding and best of all (no not the free lunches) I got to work with some great people!!! These people were on the committee, in our broader lab community and also included the diverse and wonderful people outside of the lab. The Green Man Breweries, Pasta D'oro, The University Book Shop, Velvet Burger and Custom print were some of the local businesses that answered our asking.

These business people and local identities stepped in without hesitation and helped us out. They gave prizes for our competitions during the conference..... Many of whom had absolutely no idea that when you spoke of labs techs you weren't referring to rodents in running wheels with spare ears growing on their backs. Being able to let people in our community know what we do and how important we are was probably the best part of the entire process, I made many friends and would strongly recommend getting involved if you have the chance. Show casing what we do is important, so is meeting and socializing with people doing the same job. It was an honor to be involved ... YAY for volunteering, I knew I did it for a reason!!!

So don't become an albino rat, get outside and spread the word, have some fun and open you mind ... you may also get some free food on the way.

A young lab rat from Dunedin

Barrie Edwards
21 August 1941 – 19 September 2008

The medical laboratory science community lost a fine scientist and manager, as well as a strong advocate for the profession, when we learnt of the untimely death of Barrie Edwards on the 19th September, 2008. Barrie worked at Canterbury Health Laboratories for 46 years starting as a trainee in 1961 before progressing to Charge Technologist of Haematology in 1969 and finally as their Service/ Business Development Manager from 1993 until his sudden death in September.

His professional life saw him elected to the NZIMLS Council (1972 – 1990) where for 15 years he served as secretary (1975 – 1990) and the Medical Laboratory Technologists Board (1979 – 1991) where he held the office of Deputy Chair (1986 – 1991). His contributions to the profession were many and included advocacy for a regular scientific forum with our Australian counterparts which resulted in the South Pacific Congress every four years.

Barrie has been variously described as a mentor, a great networker, and a person that was universally liked by those he came in contact with. He was someone on whom you could rely for a sound opinion as well as a positive and pragmatic response. His entrepreneurial flair was always evident and it was this talent that he utilised well over his years with Canterbury Health Laboratories. Be it in the way he promoted Canterbury Health Laboratories to the profession or the idea to sell handbags to the laboratory staff! He had a great love of family, friends and yachting – often combining all three in a social occasion - and was, in recent years, able to successfully divide his working life between Canterbury Health Laboratories and his home in the Keneperu Sound.

He is greatly missed by all his colleagues from within Canterbury Health Laboratories and across the wider New Zealand laboratory sector as well as by many who worked and socialised with Barrie over the years.

After Match Function for Barrie Edwards

Karamarina Bay, New Years Day 2009.

Picture this: a beautiful sundrenched afternoon in Karamarina Bay Keneperu Sound, a gentle northerly breeze causing the anchored yachts and runabouts at Karamarina to bounce and tug at their moorings. It was a perfect sailing day and almost two hundred guests were gathered on the lawn of the Edward's home to hold the after-match function that was always Barrie's wish and was clearly signalled at his recent funeral.

The formalities were few. At 4pm there was a compulsory toast of rum, which was followed through-out the rest of the afternoon and evening by many voluntary ones, and Jill's son Tony greeted the assembled guests. Tony mentioned that earlier in the day the family had marked the occasion by planting three commemorative Waratah trees, one at the top of the drive to greet visitors as they arrived, with another two being planted closer to the house. The brilliant red flowers of these trees will appear every year to mark the time that Barrie died.

The guests were then invited to one of Barrie's favourite spots on the beach, known by the family as "Drink Rock" where Barrie's ashes were scattered. A beautiful and spontaneous moment then just happened as each of the Grandchildren scattered a handful of Barrie's ashes at this spot. Sand was then mingled with the ashes ensuring Barrie will always be a part of his beloved beach. Many a tear was wiped from many an eye at this stage.

This sombre mood was soon overtaken with laughter as stories of Barrie's exploits, sailing, skiing and laboratory were recalled. Much was made of "spud gun" battles that took place between the Edwards and their surrounding neighbours, and Barrie's sons gave a practical demonstration of these awesome devices much to the delight of the guests, while any neighbours not present at the gathering took cover, or fired back! It was a Barrie sort of 21 gun salute!

Many people spoke of Barrie's willingness to assist others, and that nothing was ever too much trouble when friends or colleagues needed a hand.

When darkness overtook the proceedings, the remaining guests migrated toward the house, sitting or dancing on the deck while they enjoyed some of Barrie's favourite music. At the end of the evening a firework display added to the celebrations.

The poem written by the family and read at Barrie's funeral was repeated at this gathering and these verses summed up that beautiful day.

At sunset, at drinks rock
or for that evening cruise,
when having a party
and pouring the booze,
I'll be there.

In the sun on your face,
the breeze in your hair,
for a gentle walk in the bush.
I'll be there.

There can be little doubt that on that sunny afternoon at Karamarina Bay on New Years day 2009, Barrie was there.

Mike Southern
Christine Hickton

Robert (Bob) Allan. 24th March 1921 – 4th December 2008

Robert Allan, known as 'Bob' to his work colleagues, started his working life as a lab assistant in a veterinary clinic in Edinburgh and transferred from there to the Edinburgh Royal Infirmary as a technician. He was always an achiever and in the days when diplomas in two subjects were needed to qualify as a Technologist, he completed all four. He married his life-long soul mate, Ena, at Christchurch in Mornington in 1959 and their three children (Robert, Barbara and Janet) were all born in the UK. He brought his family to New Zealand for what he saw as better opportunities, but Scotland was always very close to his heart and there were many trips 'home' over the years. Each trip, like everything he did, was meticulously planned and documented. The following is an excerpt from one such trip:

"It was Plockton that we had in our sights today, for we hoped to see Hamish McBeth standing in the middle of the road, dumb and inarticulate as the Highland Beesties gathered round him. We followed the fine road by the gaping maw of Loch Linnhe, dipping round the wee inlet of Loch Creagan under the auld auld brig and over the grand new brig then up that ploutering wee twisty road that takes a body across the heid o' Loch Leven and need a body cry? Only in relief that a body is still travelling north. Our silver car skinkled in the sun, the sea glittered like diamonds and the air was so intoxicating that we had to get out and dance a wee jig!"

Bob had a great love of music and literature, and in fact completed a music degree at Otago University after his retirement from the laboratory. He had a good singing voice and was a long time, and very active member of the local Opera Company. He won his gold bar in ballroom dancing and was a delight to watch partnering a grand-daughter in a joyous reel at his sixtieth wedding anniversary. A consummate sportsman, Bob tried his hand at most things – yachting, mountaineering, skiing and in later life running. He was in his mid-fifties when he took up running and in typical Bob fashion it was not just a hobby, he was a very competitive marathon runner and completed the London Marathon with his daughter Barbara the year he turned 65. As he got older – much older – his joints got a touch of the arthritis and he switched to walking. Not short gentle strolls of course, he was often to be seen striding out many miles from home.

At home, as at work, Bob was very organised and multi-skilled. Tuesday was his cooking night. Ballet tutus for the girls, no problem, out with the trusty old sewing machine. A large vege garden, home made compost, and every ten years the house and roof got painted. It was with very real chagrin that he was obliged to get someone else in to paint the roof the last time around, although he still climbed up to inspect the quality of the workmanship. He once said he could not shift into a smaller house because there was 30 years worth of compost invested in the garden!

Bob was one of a group of technologists imported from the UK by Professor D'arth to establish a full Pathology Service at the Medical School in Dunedin. The group included Henry Shott, John Case, Derek Tingle, Brian Glynn-Jones and Derek Ford. He was involved in moving the training and education of Technologists from learning at the bench to the first formal Polytechnic course, and saw Biochemistry move from manual techniques to full automation and computerisation. Along the way he sat on the Grading Committee for many years, served as an examiner, and indulged his literary bent as Editor of the professional Journal. He was number 6 on the register with the Medical Laboratory Technologists Board when they began in 1974 and was appointed an Oral Examiner in 1979. He was an excellent lecturer with an in-depth knowledge of his subject and a prodigious memory for complexities like the structure of the porphyrin ring. Within the Laboratory as Charge Technologist in Biochemistry he was a firm but fair boss who was both loved and respected by the staff. When the laboratory was relocated from the Medical School to the new Clinical Services building in the late 60's he was largely responsible for the planning of the new facility which has only recently been rebuilt. As with everything he did, it was carefully planned and nothing was forgotten or left to chance.

In retirement, while he did not often visit the laboratories, he maintained a keen and close interest in the profession and kept ongoing contact with many of his erstwhile colleagues. This included attending both Dunedin Hospital reunions and participating as part of a very talented quartet.

Bob not only achieved so much himself, he also encouraged us lesser mortals to reach for the stars. It was a long life, well lived, and the world is a richer place for his having passed this way.

Haematology Special Interest Group

Journal Article Questionnaire

Article: "The classification and diagnosis of erythrocytosis"
M.F.McMullin
International Journal of Laboratory Hematology, December 2008, Vol 30, Issue 6. p 447 – 459.

Questions

1. What FBC parameter most accurately reflects blood viscosity?
2. In what situation, and how can this accuracy be affected?
3. In what patient type may measured and predicted red cell mass measurement (RCM) be inaccurate?
4. Using RCM predicted values define an "absolute" and "apparent" erythrocytosis.
5. An absolute erythrocytosis can be assumed from what Hct values?
6. Erythrocytosis is classified into three main groups, what are they?
7. What genetic mutation has been demonstrated in the majority of patients with polycythaemia vera (PV)?
8. Erythropoietin (EPO) levels in PV patients is expected to be low/high?
9. Secondary erythrocytosis is classified into two main groups, what are they?
10. Name the six causes of congenital secondary erythrocytosis.
11. How many EPO receptor mutations have been described?
12. How are high oxygen affinity Hb's best detected, and what is the expected result?
13. What other characteristic may high oxygen affinity Hb's have?
14. Can Hb electrophoresis detect these variants?
15. What does VHL stand for?
16. What is the Chuvash mutation?
17. Acquired erythrocytosis is classified into 4 groups, what are they?
18. High altitude and carbon monoxide poisoning are examples of which group of acquired erythrocytosis.
19. What percentage of renal transplant recipients can develop erythrocytosis?
20. Idiopathic erythrocytosis is divided into two groups, what are they?
21. What has allowed revision and simplification of diagnostic criteria for PV?
22. What two findings are sufficient to make a diagnosis of PV?
23. When investigating an erythrocytosis, what test should be done following a negative JAK2 and why?

Questions compiled by Jacquie Case, Special Haematology section, Middlemore Hospital, Auckland. Contact details for a copy of the journal; Ph (09) 276 0044, ext: 8515. e-mail: jcase@middlemore.co.nz

Answers on page 33

Journal questionnaire

Below are 10 questions based on articles in the April 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. Due to a number of members experiencing problems in submitting it is recommend that you write your answers in a word doc and then cut & paste your answers on the web site.

The site will remain open until Friday 12th June 2009. You must get a minimum of 8 questions right to obtain 5 CPD points.

Journal questions

1. What biological phenomena does laminin participate in.
2. At which anatomical site does laminin deposition determine the formation of a true basement membrane along sinusoids and what is this phenomenon called.
3. What were the sensitivities, specificities, positive predictive values and negative predictive values for serum laminin before treatment and after treatment.
4. What mechanisms are proposed for the elevation of serum laminin concentrations in chronic hepatitis patients.
5. By what mechanism do the authors think that the gradual decrease in serum laminin after the beginning of treatment occurs.
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.
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.
8. What did the authors consider the advantage and major advantage of the DM96 to be.
9. What did the authors state what the recurrent source of error was.
10. What did the authors conclude that the results of their study have demonstrated.

Questions and answers for the November 2008 Journal questionnaire

1. What is the typical blood film appearance in Homozygous Hb E patients and what other condition is it similar to. **Hypochromia, microcytosis & presence of target cells. Similar to beta thalassaemia trait.**
2. What are the usual levels of Hb F in Hb E/ß^0 thalassaemia and what range of levels have been documented in the literature. **30-60%. 5-87%.**
3. What may prove helpful in detecting a beta thalassaemia mutation. **Sequencing of the globin gene.**
4. Why is a correct diagnosis of Hb Eß^0 thalassaemia important. **Disease monitoring and treatment of the patient, and in regard to reproductive choices.**
5. At what percentage are reticulocytes normally present in the peripheral circulation and what can enhance their release. **About 1% of the red cells. Intense erythropoetic stimulation.**
6. In the quoted study by the Spanish Multicentric Study Group what was haematopoietic recovery indicated by. **ANC of >0.5 x 10^9/L or an INF >5% or HFR >3%.**
7. What was the diagnosis and treatment of the paediatric patients in the study by Yeu-Sheuan Khor. **Malignant lymphoma, primary thrombocytopenia, neuroblastoma. Autologous PSC infusion, allogenic CB infusion, autologous HPC & marrow infusion.**
8. How many and what percentage of patients had an immature reticulocyte fraction of ≥5% and what parameter at which level was this fraction preceded by. **6 out of 7, 86%. ANC of +- 0.5 x 10^9/L.**
9. In Ross Hewett's viewpoint article what does he perceive to be the biggest challenge for anyone working in a laboratory. **Managing fellow scientists.**
10. Where and when is the next NZIMLS Annual Scientific Meeting and what is its theme. **Blenheim, 24-28 August 2009 (or 17-20 August 2009), Sauvignon and Science.**

Editor's note: when the Journal was printed the dates of 24-28 August were correct. Subsequently, due to a clash with another scientific meeting in Christchurch the ASM was rescheduled to a week earlier. Either answers were deemed correct.

Med-Bio Journal Award

med·bio

Med-Bio 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.

Winners of the Med-Bio Journal Award from the November 2008 issue was Jaine Duncan, Haematology, Canterbury Health Laboratories for her article "A retrospective review of homozygous Haemoglobin E patients". *N Z J Med Lab Science 2008; 62(3): 61-62*

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.

Joint winners of the NZIMLS Journal Prize for 2008 were Sarla Naran and Shards Lallu, Department of Cytology, Anatomic Pathology, Wellington Hospital for their article "Pulmonary mucormycosis diagnosed by brushing cytology. A case report". N Z J Med Lab Sci 2008; 62 (2): 32-34.

The judges commented that this was a well written and interesting case study, had good use of figures and the discussion was structured and informative.

Just the Job – Medical Laboratory Science

JUST THE JOB

The Council of the New Zealand Institute of Medical Laboratory Science is pleased to announce, as part of its commitment to promote the profession, the screening of a segment on Medical Laboratory Science on the "Just the Job" TV series. This will be aired on TV2 Saturday 9th May at 9.30am with two other healthcare professions in this episode.

The NZIMLS has been working with Dave Mason, the producer of the series in the making of this segment, and committed time and funds to its final production.

Every Laboratory in New Zealand will be gifted a copy of the DVD by council via the regional representatives. More copies are available and will help each Lab with any promotional activities within their area.

The NZIMLS will use the DVD with its activities at Career Expo's in 2009 and will also give copies to AUT, Massey and Otago Medical Laboratory Science Schools.

From the website www.davemason.co.nz

"Just the Job is a TV series giving high school students a unique up-close and personal insight into the myriad of career paths on their doorstep and elsewhere in New Zealand. We all know just how difficult it can be deciding exactly what career might suit us best. That's where Just the Job steps in...

Each series of 10 half hour episodes features three careers each programme, the series has been designed as an informative and entertaining tool to help secondary school students gain a first hand understanding of what happens "on the job".

Each week, students from the 14 to 18 year old age range try out their possible future careers. By adding a mentor and some hands-on experience into the mix they have every chance to find out the pro's and con's of the job before deciding whether it's for them or not.

The series not only creates awareness of a variety of careers but shows what is available in one part of the country to young career seekers in another part of the country. Just the Job helps students realise they have a raft of opportunities available to them and that they can have exciting, challenging careers if they just know where to look and get started.

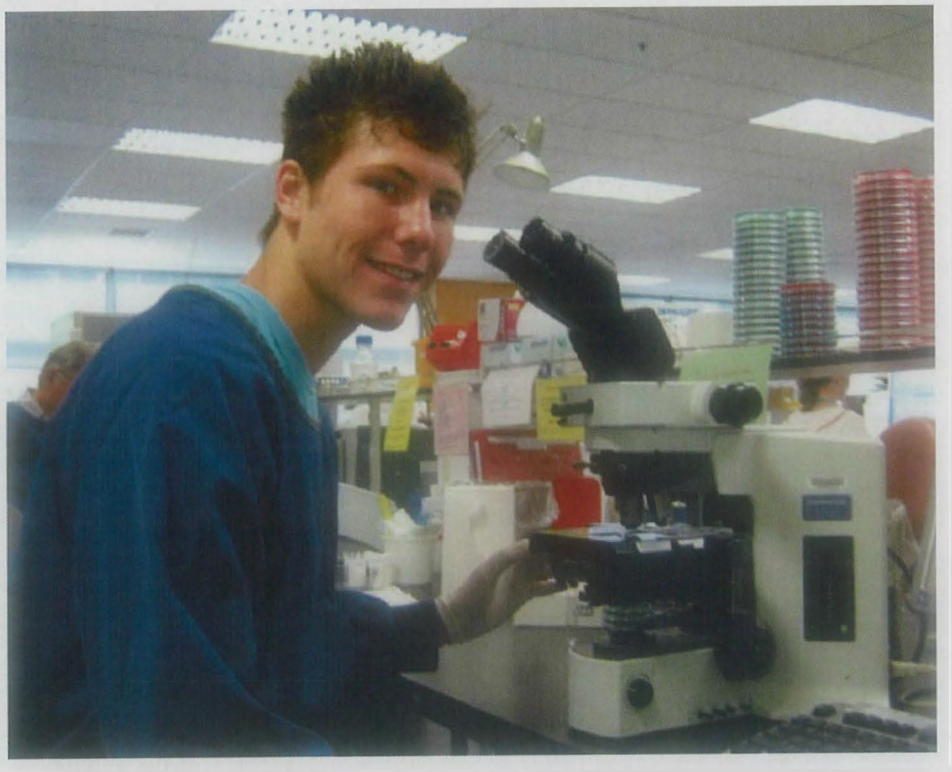
Clinton Randell, a NZ Idol top 10 finisher, presents the lively introductions to each segment. The great aspect of this programme is it demonstrates there is a lot more to particular careers than people may have ever thought. Often preconceived ideas are just blown out of the water.

Career Services Advisors, Sarah McIndoe and Selwyn Insley wrap up each episode with some expert advice designed to help high school scholars kick start their own career investigations."

Synopsis

Just the Job - Series 3 - Episode 10 – To be Aired TV2 - Saturday 9th May 2009 at 09.30am

Just the Job wraps up another successful series this week with a special show featuring a number of exceptional career opportunities in the medical profession. You don't need to become a doctor to enjoy a medical career – there are many other career paths offering challenge, variety and a rewarding way to help others, as you'll see in this medical special.



Hospital and Operating Theatre

Community Healthcare

Medical Laboratory Scientist

It's one of the unseen sciences but in this story, join Wynand as he gets to find out some of the fascinating work that goes on at Labplus. If you love sciences and solving puzzles, then this could be just the job for you. Wynand meets Kristen who gives him an introductory look at what a medical laboratory scientist does. He finds out there are many different aspects to the role (which requires a degree) and samples that need testing and analysing come in many different forms.

Kristen shows Wynand cells under the microscope and explains what is unusual and what the unusual element means. The first sample they investigate shows malaria and Kristen deduces the person has recently returned from overseas where they would have contracted the illness.

Demand for people in this career is high so if Wynand decides to pursue this occupation, he has excellent chances of securing a great job that ultimately helps others.

We hope NZIMLS members appreciate this initiative the NZIMLS council is making on your behalf in raising the profile of Medical Laboratory Scientists and encouraging high school leavers to pursue a career in our profession.

Ross Hewett.

News from the PPTC

New Staff Member

Phil Wakem has been appointed as the PPTC's Programme Coordinator. Prior to his appointment to the PPTC, Phil was already known to many of the laboratory staff from the Pacific through his involvement in the Haematology courses run here at the PPTC and also as the co-ordinator of the Haematology EQA surveys. He is very enthusiastic and passionate when it comes to the teaching of haematology and he has already been in contact with all the laboratories in the Pacific regarding enhancements to the Haematology teaching programme. With Phil 'on-board' it will give us the opportunity to expand our training programme and we are looking at running courses in-country. We have discussed this with NZAID, our principle funders, and they also are enthusiastic about us conducting training courses in-country as well as here at the PPTC. It is great to have Phil as part of the PPTC Team and so keep a look-out for these new initiatives.

Training Courses for 2009

We have received funding from WHO to repeat the very successful distance learning programme that was run through WHO's POLHN website over the last two years leading to the PPTC's Diploma in Medical Laboratory Technology. We are very pleased to report that 20 students from Pacific Island Countries have successfully graduated with this DipMLT and they will be receiving their Diplomas very shortly. A number of lab staff have only one or two of the modules left to complete and so it is hoped that they will complete these during this cycle that commences with Biochemistry at the beginning of March. It is anticipated, that commencing in 2010 this Diploma course will be upgraded and will include a significant amount of practical work that will have to be completed by the student in their own laboratory. This work will be supervised by their Charge Technologist and signed off in a logbook.

The students who graduated with the Diploma in 2008 are:

Tonga		**Kiribati**	
Timote	Fakasi'eiki	Tiaon	Tongaibeia
Mele Vea	Fonua	Itaaken	Barieti
Fele'unga	Vaka'uta	Boutu	Berenateta
Sokopeti	Iketau		
Viela	Tapa'atoutai	**Yap**	
Falekakala	Tomu	Paula	Matyed
Aiona	Ha'unga	Maria	Marfel
		Lucy	Dibay
Palau		Elizabeth	Namgey
Catherine	Ksano		
March	Kloulubak	**Cook Islands**	
April Lynn	Solang	Victoria	Wuatai
Romuel	Loquinario	Romuel	Loquinario
Evelyn	Tomokane	Evelyn	Tomokane

Courses to be run at the PPTC during 2009 are; Blood Cell Morphology in April/May, Blood Bank Technology in September and Microbiology in November.

WHO Strategy for Strengthening Health Laboratory Services

A meeting of significant importance to laboratories in the Pacific Region was held at WHO Manila in early December. This meeting reviewed a document called "Asia-Pacific Strategy for Strengthening Health Laboratory Services [2010 – 2015]". In its introduction the document states:

Laboratories are an essential and fundamental part of all health systems and their goals to improve health. Reliable and timely results from laboratory investigations are crucial elements in decision making in almost all aspects of health services. This ranges from critical decisions concerning health security, national economies and meeting obligations such as IHR to equally vital decisions about the health and well being of individuals.

When finalised, this strategy document will be put to the WHO Regional Meeting later this year. If approved, it will mean that every country in the region is committed to introducing a national laboratory policy to cover all aspects of their laboratory services including adequate resourcing and the setting up of national standards. We will be hearing lot more about this in the next few months.

Visit to Fiji

Early in December, John and Phil visited Fiji for discussions with WHO and Fiji School of Medicine on training programmes. A distance learning course on Laboratory Diagnosis of STIs including HIV is being planned for teaching through POLHN commencing early 2010. This course will also include laboratory workshops conducted in the student's laboratories.

During this time in Fiji we also visited laboratories in Suva, Nadi and Lautoka, and Phil conducted a blood film training session in Lautoka. It was a good chance to introduce Phil and for him to meet lab staff.

Retirement

We have just recently heard that Catherine Betiei Ksano from the laboratory in Palau has retired. Catherine has worked in the lab in Palau for many years and attended courses here at the PPTC. We wish her all the very in her retirement.

Catherine is second from left in the front row of this PPTC class photo of 1994

Abstracts of articles in the British Journal of Biomedical Science

Liu XS, Zhang XQ, Tian T, Liu L, Ming J. Influence of homeobox B2 antisense oligodeoxynucleotides on the biological characteristics of in vitro cultured primary human umbilical vein endothelial cells. *Br J Biomed Sci 2008; 65(1): 22-7.*

This study aims to explore the influence of homeobox B2 (HOXB2) antisense oligodeoxynucleotides (asodn) on the biological characteristics of in vitro cultured primary human umbilical vein endothelial cells (HUVECs). The distribution of HOXB2 asodn in the HUVECs was observed by fluorescent labelling, and the influence of different concentrations of HOXB2 asodn on the DNA synthesis of HUVECs was assessed. Flow cytometry and a reverse transcriptase-polymerase chain reaction (RT- PCR) method were employed to observe the influence of HOXB2 asodn on HOXB2 expression and the HUVEC cell cycle. After the induction of liposome, the nuclear fluorescent staining of HOXB2 asodn was weaker 15 min after transfection and the staining reached the strongest level at 4-8 h but then weakened and disappeared by 16 h after transfection. This indicated that endothelial DNA synthesis could be inhibited by HOXB2 asodn in a dose-dependent manner. Furthermore, the HUVECs could be delayed in their passage from G1 to S. Simultaneously, expression of HOXB2 mRNA had decreased significantly by 24-48 h after transfection. Clearly, HOXB2 plays important roles in the proliferation of endothelial cells and also affects the cell cycle.

Clarke L, Moore JE, Millar BC, Crowe M, Xu J, Goldsmith CE, et al. Molecular epidemiology of *Pseudomonas aeruginosa* in adult patients with cystic fibrosis in Northern Ireland. *Br J Biomed Sci 2008; 65(1): 18-21.*

Isolates (n = 51) of *Pseudomonas aeruginosa* obtained from the sputa of 29 adult patients attending the Regional Cystic Fibrosis Centre in Northern Ireland were compared using an enterobacterial repetitive intergenic consensus sequence (ERIC2) primer in a random amplification of polymorphic DNA (RAPD) polymerase chain reaction (PCR) method. Resulting banding patterns showed a high degree of genetic heterogeneity among all isolates from the patients examined, suggesting a non-clonal relationship between isolates from these patients, when employing this genotyping technique.

Davies S, McIntyre S, Fowler J, Tovey M. Methicillin-resistant *Staphylococcus aureus* detection using chromogenic media: the Sheffield experience. *Br J Biomed Sci 2008; 65(1): 13-7.*

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to cause major problems, both in hospitals and the community. Microbiology departments need to review their methodology regularly to ensure that they are contributing in the most appropriate manner to the battle against MRSA. Media employing chromogenic enzymes to aid the isolation and identification of MRSA is a relatively new approach. In this study, 192 swabs from 112 different patients were inoculated on two chromogen-containing media and four other commonly used solid MRSA media to determine which gave the appropriate combination of sensititivity, specificity and speed of result. Methicillin-resistant *S. aureus* was isolated on at least one of the six media from 102 of the 192 swabs. Both chromogenic media proved to be statistically significantly more sensitive than the other media after overnight incubation and had a sensitivity of 96% after 48 hours' incubation. The recent introduction of chromogen-containing MRSA media offers microbiology laboratories the opportunity to isolate and confirm the majority of MRSA infections/colonisations in 24 hours, which should result in better patient care. The possible slight increase in costs should not provide a valid excuse for using inferior methodologies.

Barber R. Evaluation of the BD ProbeTec ET system for the direct detection of *Mycobacterium tuberculosis* from clinical samples. *Br J Biomed Sci 2008; 65(1): 7-12.*

Early detection of disease caused by members of the *Mycobacterium tuberculosis* complex (MTBC) is essential in controlling transmission of tuberculosis. Clinical evaluation of the BD ProbeTec ET system for the direct detection of MTBC organisms in clinical specimens is carried out by comparing results obtained with conventional liquid culture and reference laboratory identification. In 186 specimens (159 respiratory, 27 non-respiratory), the BD ProbeTec ET assay detected eight out of 11 MTBC culture-positive specimens, with overall sensitivity, specificity, positive predictive value and negative predictive value of 85%, 100%, 100% and 99%, respectively. The data show that the BD ProbeTec ET assay is a highly sensitive and specific technique, and gives an accurate result from a clinical specimen within three to four hours.

Hannon-Fletcher MP, Barnett YA. Lymphocyte cytochrome P450 expression: inducibility studies in male Wistar rats. *Br J Biomed Sci 2008; 65(1): 1-6.*

The cytochrome P450 system plays a key role in the metabolism of endogenous and exogenous compounds. The system is distributed widely in body tissues, with the highest concentration of the enzymes found in liver hepatocytes. Extrahepatic expression of the P450 system has been documented in the lung, pancreas and kidney, and the enzymes are induced by many disease states, including diabetes mellitus and cancer. Little attention has been paid to the expression and inducibility of the system in peripheral blood lymphocytes. In this study, specific P450 inducers are administered in vivo to male Wistar rats. The expression and in vivo induction of the P450 isoforms CYP2B, CYP2E, CYP3A and CYP4A in liver and lymphocyte samples is determined using Western blot analysis. Following in vivo induction, the lymphocyte P450 proteins showed an average three-fold increase in expression (0.003-0.005 microg P450/microg microsomal protein), compared to the control lymphocyte samples. Expression in the induced lymphocyte samples was up to 11-fold lower than that in the induced liver samples, as expected. These results indicate that lymphocytes may provide a relatively simple method by which to monitor the P450 profile in human subjects.

Hardy SR, Watson FR. Early municipal bacteriology in Brighton, Aberdeen and Bristol: blessing or burden? *Br J Biomed Sci 2008; 65(2): 109-18.*

In contrast to the idea that bacteriology was introduced as a tool for the diagnosis and management of the individual patient, this review highlights the work of the municipal bacteriological laboratory in the United Kingdom to illustrate how bacteriological laboratories were introduced as means to control community epidemic disease. Using the examples of municipal laboratories in Brighton, Bristol and Aberdeen, it shows how public health considerations of community infectious diseases such as diphtheria and typhoid dominated the early development and workload of the municipal laboratory, rather than examination of patients with pathological states of uncertain aetiology. It argues that this public health focus of the Medical Officer of Health limited the range of diagnostic tests carried out in such laboratories for over two decades. The growing number of pathogenic microbes being discovered in the late 19th century appears to have had little impact on the tests being carried out in the municipal

laboratory. Municipal bacteriological facilities in three towns, a central municipal laboratory (in Brighton), a central university pathological department (Aberdeen) or a combination of both (Bristol) all provided the same limited set of tests. This restricted set of bacteriological examinations is likely to have diminished the value and status of bacteriology in what should have been a period of increasing scope.

Shitara M, Tsuboi Y, Sekizuka T, Tazumi A, Moorei JE, Millar BC, et al. Genetic heterogeneity of the dnaK gene locus including transcription terminator region (TTR) in *Campylobacter lari. Br J Biomed Sci 2008; 65(2): 95-101.*

Nucleotide sequences of approximately 3.1 kbp consisting of the full-length open reading frame (ORF) for grpE, a non-coding (NC) region and a putative ORF for the full-length dnaK gene (1860 bp) were identified from a urease-positive thermophilic Campylobacter (UPTC) CF89-12 isolate. Then, following the construction of a new degenerate polymerase chain reaction (PCR) primer pair for amplification of the dnaK structural gene, including the transcription terminator region of C. *lari* isolates, the dnaK region was amplified successfully, TA-cloned and sequenced in nine C. *lari* isolates. The dnaK gene sequences commenced with an ATG and terminated with a TAA in all 10 isolates, including CF89-12. In addition, the putative ORFs for the dnaK gene locus from seven UPTC isolates consisted of 1860 bases, and the four urease-negative (UN) C. *lari* isolates included C. *lari* RM2100 reference strain 1866. Interestingly, different probable ribosome binding sites and hypothetically intrinsic p-independent terminator structures were identified between the seven UPTC and four UN C. *lari* isolates, respectively. Moreover, it is interesting to note that 20 out of a total of 28 polymorphic sites occurred among amino acid sequences of the dnaK ORF from 11 C. *lari* isolates, identified to be alternatively UPTC-specific or UN C. *lari*-specific. In the neighbour-joining tree based on the nucleotide sequence information of the dnaK gene, C. *lari* forms two major distinct clusters consisting of UPTC and UN C. *lari* isolates, respectively, with UN C. *lari* being more closely related to other thermophilic campylobacters than to UPTC.

El-Abd E, El-Tahan R, Fahmy L, Zaki S, Faid W, Sobhi A, et al. Serum metastasin mRNA is an important survival predictor in breast cancer. *Br J Biomed Sci 2008; 65(2): 90-4.*
This study investigates the possible prognostic role of serum metastasin messenger RNA (mRNA) in breast carcinoma as a non-invasive screening tool, and determines metastasin mRNA in the serum of breast cancer patients with high sensitivity (85%) and specificity (100%). A significant difference (P = 0.05) was observed between serum metastasin mRNA and the number of involved lymph nodes. Patients with higher expression of serum metastasin showed poor survival (six times worse) than those with lower levels. Patients negative for serum metastasin mRNA suffered recurrences, while those positive for serum metastasin mRNA suffered distant metastases. The results of this study suggest that serum metastasin mRNA represents an important survival marker in breast carcinoma.

Crowley D, Cryan B, Lucey B. First detection of a class 2 integron among clinical isolates of *Serratia marcescens. Br J Biomed Sci* **2008; 65(2): 86-9.**
Serratia marcescens is a frequent nosocomial isolate and is associated with a variety of clinical sources, including blood, urine and sputum, and can cause significant infection. Infections can be difficult to treat due to its resistance to a variety of antimicrobial agents. An investigation of a population of 30 clinical strains revealed the presence of a class 2 integron among nine of the isolates, which represents the first isolation of this integron in Serratia species. This integron contained the gene cassettes dfrA1, sat1 and aadA1, conferring resistance to trimethoprim, streptothricin and streptomycin/ spectinomycin, respectively. One of these isolates also carried a class 1 integron identified by sequence analysis as containing the open reading frames aacC1 (encoding gentamicin resistance), ORFX, ORFY and aadA1. Polymerase chain reaction analysis confirmed the presence of the qac epsilon delta1 and sul1 markers, which are common among class 1 integrons.

George S, Chaturvedi P. Protective role of Ocimum canum plant extract in alcohol-induced oxidative stress in albino rats. *Br J Biomed Sci* **2008; 65(2): 80-5.**
Ethanol is the most frequently abused drug and causes a variety of pathological disturbances. It causes toxicity to tissues by generating free radicals during the course of its metabolism that can damage cellular structure and function, especially in hepatocytes. This study investigates the preventive and protective effects of *Ocimum canum* on alcohol-induced oxidative stress. Male Wistar rats were used in three separate experiments. First, two groups of six rats each (normal control and alcohol-treated) were used to establish hepatotoxicity. The alcohol-treated group showed a significant increase in TBARS and decreased activities of SOD, catalase, GSH, alpha-tocopherol and ascorbic acid. Second, the preventive effect of the *O. canum* extract was assessed. Four groups of rats (six in each group) were used and the experimental groups were treated with ethanol and graded doses of the extract for four weeks. Normal control and alcohol-treated groups were also assessed. Lipid peroxidation and antioxidant potential were quantified in plasma samples, which showed that the extract had a preventive effect. Third, the curative effect of the extract was assessed. The rats were divided into four groups comprising a normal control group on a normal diet and three other groups given alcohol for four weeks to establish alcohol toxicity. One of the alcohol groups was used as a control and the other two alcohol groups were given graded doses of the extract. After four weeks the rats were sacrificed in order to assess the lipid peroxidation and antioxidant potentials. The results indicated that the *O. canum* extract had hepatoprotective abilities against alcohol-induced oxidative stress.

El-Nabi Kamel MA, Shehata M. Effect of toluene exposure on the antioxidant status and apoptotic pathway in organs of the rat. *Br J Biomed Sci* **2008; 65(2): 75-9.**
The chronic abuse of the solvent toluene results in structural and functional impairment of various organs. However, the pathophysiological mechanisms that cause these impairments of function are not clearly understood. This study aims to assess the effect of chronic toluene exposure (15, 30 and 45 days) on the oxidative stress and antioxidant status of different organs in the rat. Also, cyclooxygenase-2 and caspase-3 activities (a marker of apoptosis) are studied. Forty male albino rats were used and divided into four groups: controls (group I) and three other groups receiving a single daily dose of toluene (650 mg/kg) for 15 days (group II), 30 days (group III) and 45 days (group IV). The animals were then sacrificed and the brain cortex, cerebellum, liver, kidney and testis were separated for the determination of thiobarbituric acid reactive substance (TBARS), GSH, glutathione disulphide (GSSG) and glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), cyclooxygenase-2 (COX-2) and caspase-3 activity. Results showed a significant and time-dependent increase in the levels of TBARS, GSSG and in GST, SOD, COX-2 and caspase-3 activity, while GSH, GR and GPx showed a marked decline in most tissues. The brain (cortex and cerebellum) was the most affected organ, showing the greatest increase in one apoptotic marker (caspase-3), while the testis and kidneys were least affected. In conclusion, oxidative stress and derangement of the GSH:GSSG ratio, induced chronic inflammatory change and apoptosis may play an essential role in toluene toxicity.

Whiting PH, Kalansooriya A, Holbrook I, Haddad F, Jennings PE. The relationship between chronic glycaemic control and oxidative stress in type 2 diabetes mellitus. *Br J Biomed Sci 2008; 65(2): 71-4.*

This study compares the lipid peroxidation marker urinary thiobarbituric acid reactive substances (TBARS) and antioxidants including plasma alpha-tocopherol (vitamin E), plasma (P-GSH-Px) and erythrocyte glutathione peroxidase (E-GSH-Px) activities, and plasma selenium levels in two groups of type 2 diabetic subjects (both n=20) with a disease duration of < or =2 (GP1) and 4-6 years (GP2), and non-diabetic age and gender-matched control subjects (CG, n=20). The mean (standard deviation [SD]) age of the groups was similar at 41(10) years. Fasting blood and midstream urine samples were obtained from diabetic and non-diabetic subjects attending the diabetic clinic and HbAlc, fructosamine, urine TBARS, total antioxidant (TAS) levels, P-GSH-Px, E-GSHPx and plasma selenium and vitamin E concentrations were measured. HbA1c (%) and fructosamine levels in the GP1 and GP2 diabetic subjects and the controls were 5.75 (0.67), 11.43 (2.01) and 4.33 (0.47), and 3.09 (0.57), 6.09 (1.15) and 1.67 (0.31), respectively (GP1 vs. GP2, GP1 vs. GC and GP2 vs. CG, all $P < 0.001$). Elevated urinary TBARS (micromol/mmol urinary creatinine) in the GP1, GP2 and GC groups were 2.47 (0.37), 3.73 (0.93) and 1.18 (0.24), respectively (GP1 vs. GP2, GP1 vs. GC and GP2 vs. CG, all $P < 0.001$). A significant correlation between HbA1c and TBARS was also noted ($r2 = 0.894$, $P < 0.001$) but only in the GP2 subjects. TAS levels were only decreased in the GP2 group compared to control values (0.59 [0.18] vs. 1.74 [0.21], $P < 0.001$). Plasma vitamin E concentrations (micromol/L) of 34.11 (3.31), 9.57 (2.20) and 39.01 (2.91) were observed in the GP1, GP2 and GC groups, respectively (GP1 vs. CG, $P < 0.05$ and GP1 vs. GP2 and GP vs. CG, both $P < 0.001$). E-GSH-Px (U/g Hb) and P-GSH-Px (U/L) activities in GP1, GP2 and CG groups were also decreased at 57.04 (4.31), 24.0 (8.94) and 67.6 (4.29), and 6.16 (1.56), 2.67 (0.47) and 8.72 (0.31), respectively (E-GSH-Px: CG vs. GP1, $P < 0.01$, CG vs. GP2 and GP1 vs. GP2, both $P < 0.001$; P-GSH-Px: CG vs. GP1, CG vs. GP2 and GP1 vs. GP2, all $P < 0.001$). Plasma selenium levels (miromol/L) were only significantly decreased in GP2 compared to both GP1 and CG values (0.49 [0.29] vs. 1.67 [0.80] vs. 1.79 [0.26], both $P < 0.001$). These observations support the suggestion that chronic hyperglycaemia can influence the generation of free radicals, which may lead ultimately to increased lipid peroxidation and depletion of antioxidants, and thereby enhanced oxidative stress in subjects with type 2 diabetes mellitus.

Orchard GE, Torres J, Sounthararajah R. Use of softening agents to improve the production of formalin-fixed, paraffin-embedded sections of nail tissue: an assessment. *Br J Biomed Sci 2008; 65(2): 68-70.*

The use of tissue softeners to enhance the quality of tissue sections of heavily keratotic tissue is not widely published. There are very few indicators in the scientific literature that attempt to compare and contrast the benefits and disadvantages of such techniques, as most are passed down through word of mouth rather than through published data. This study attempts to present a preliminary evaluation of several methods employing tissue softeners to facilitate the preparation of reproducible, good-quality formalin-fixed, paraffin-embedded sections of nail tissue. A standard 10-minute surface application of each softener is employed for all paraffin-embedded tissue in order to ensure consistency. The results show that the use of Veet (hair remover), Fairy Liquid or fabric conditioner provides the most beneficial results. Thus, widely available products can be used in preference to specific commercially produced reagents that have no clear benefits and can cost considerably more to purchase. This study will form the basis of a more in-depth evaluation of the most beneficial softeners, in an attempt to determine optimal parameters for their use in routine histopathology laboratories.

Fujimori F, Shimizu T, Takada T, Narita J, Suzuki E, Gejyo F. Differences in lymphocyte profile between BAL fluid and human lung tissue from patients with interstitial lung disease. *Br J Biomed Sci 2008; 65(2): 63-7.*

Bronchoalveolar lavage (BAL) is a technique that samples the inflammatory cells from distal airways and alveoli; however, it is unclear whether or not cellular profiles in the BAL fluid reflect the cellular components of the lung parenchyma in interstitial lung disease (ILD). The aim of this study is to compare immunophenotypes of lymphocytes between BAL fluid and human lung tissue from patients with ILD. Fourteen consecutive patients with ILD who underwent BAL and surgical lung biopsy were enrolled. The diagnosis of ILD was confirmed by the presence of clinical symptoms and impaired respiratory function and on high-resolution computed tomography (CT) of the chest. Mononuclear cells in BAL were immunophenotyped for the expression of CD3, CD4, CD8, CD19, CD45, and CD103 by flow cytometry. Lung tissue obtained by surgical biopsy was digested with collagenase and then centrifuged to extract parenchymal cells. Isolated cells were also immunophenotyped for the same CD expression. Frequencies of positive cells were compared statistically between BAL and different lobes. Seven out of 14 patients were diagnosed clinically as suffering idiopathic interstitial pneumonia. Frequency of CD19+ cells from BAL was significantly lower than that from the upper/middle lobes ($P < 0.05$). Frequency of CD103+ cells from BAL was significantly higher than that from the upper/middle lobes and the lower lobe ($P = 0.01$ and $P < 0.05$, respectively). Comparison between different lobes demonstrated that the frequency of CD4+ cells from the upper/middle lobes was significantly lower than that from the lower lobe ($P < 0.05$). The results suggest that lymphocyte immunophenotype profiles from BAL may not reflect those in the inflammatory tissue of ILD.

Kakinuma Y, Iida H, Sekizuka T, Taneike I, Takamiya S, Moore JE, et al. Molecular characterisation of urease genes from urease-positive thermophilic campylobacters (UPTC). *Br J Biomed Sci 2008; 65(3): 148-52.*

This study aims to clarify the molecular characteristics of the urease gene operon from urease-positive thermophilic campylobacters (UPTC) obtained from different sources and in various countries. Sequence heterogeneity was observed for the promoter structures at the -35-like region among the 12 isolates examined. The most probable TTG start codon was suggested for the ureB and ureH genes, and for the ureA, E, F and G genes, ATG was suggested among all the isolates examined. Overlap was detected between ureA and ureB and between ureB and ureE among all the isolates examined. UPTC is the first example of an overlap between the two structural genes ureA and ureB. When the completely sequenced open reading frames (ORFs) for ureE, ureF, ureG and ureH were identified, non-coding regions between ureE and ureF, ureF and ureG, and ureG and ureH were also demonstrated. All six start codons of the six urease genes were demonstrated to be preceded by Shine-Dalgarno sequences among all the isolates examined. The Cys-His sequence corresponding to urease active sites were aligned perfectly and fully conserved among the three UPTC isolates examined. A putative and intrinsic p-independent transcriptional terminator was identified to be identical among all the isolates examined. A partial and putative ORF of about 200 bp in length showing high sequence similarity to GTP cyclohydrolase I was observed downstream of ureH.

Denny BJ, West PW, Mathew TC. Antagonistic interactions between the flavonoids hesperetin and naringenin and beta-lactam antibiotics against Staphylococcus aureus. *Br J Biomed Sci 2008; 65(3): 145-7.*

Flavonoids are a group of polyphenolic plant compounds with a range of biological activities. This study shows that the flavonoids hesperetin and naringenin have antibacterial activity against methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolates. Minimum inhibitory concentrations for hesperetin were 250 and 500 microg/mL, respectively, and for naringenin were 125 and 250 microg/mL, respectively. This effect was reversed by the beta-lactam antibiotics methicillin, penicillin and oxacillin, but not by cefoxitin. For bacteria growing in the presence of these antibiotics, the flavonoids had no effect on the levels of beta-lactamase enzymes and PBP-2′ compared to controls. Electron

microscopy showed abnormal morphology in bacteria treated with subinhibitory concentrations of flavonoids. These results are interesting because previous studies have reported synergistic interactions between flavonoids and beta-lactam antibiotics. It is suggested that an interaction removes both inhibitors from the bacterial growth milieu.

Davies S, Mcintyre SM, Mckinven A. Evaluation of a new *Staphylococcus aureus* latex agglutination kit, Prolex Staph Xtra, against other third-generation kits. *Br J Biomed Sci* **2008; 65(3): 142-4.**

Staphylococcus aureus, including methicillin-resistant strains, continues to be a common cause of infection/colonisation, which necessitates accurate and prompt diagnosis in the laboratory. Several rapid agglutination tests that aid this function are available, and some have been modified to improve their performance. One such kit, Prolex Staph Xtra, has been released recently. This study aims to compare this kit with other improved kits (i.e., Pastorex Staph-Plus, Staphaurex Plus and Staphytect Plus) and investigate their ability to confirm the identity of 100 strains of *S. aureus.* Results showed that 50 were resistant to methicillin. Specificity was checked against 30 strains of coagulase-negative staphylococci and 20 Enterococcus species isolates. Of the four kits tested, Prolex Staph Xtra and Pastorex Staph-Plus proved superior in terms of sensitivity and speed.

Nwose EU, Richards RS, Kerr RG, Tinley R, Jelinek H. Oxidative damage indices for the assessment of subclinical diabetic macrovascular complications. *Br J Biomed Sci 2008; 65(3): 136-41.*

Subclinical cardiovascular disease (SCVD), including complications in diabetes, is associated with oxidative damage and precedes future cardiovascular disease (CVD). Hence, assessment and management of oxidative damage is imperative. This study investigates biomarkers associated with CVD, diabetes and oxidative stress in order to determine a set of indices that could be useful to assess oxidative damage in diabetic macrovascular pathogenesis. A total of 266 participants were selected and divided into seven groups (control, family history of diabetes, prediabetes, prediabetes with CVD, diabetes mellitus [DM], DM+CVD and CVD) based on clinical history/status. Blood glucose (BG) level, erythrocyte glutathione (GSH), malondialdehyde, methaemoglobin, D dimer, homocysteine, blood viscosity and cholesterol profile were determined. Factorial MANOVA and independent univariate analyses were performed. Prevalence of significant biomarkers was assessed following a 3.5-year retrospective study. Multivariate analysis showed statistically significant differences between groups ($P < 0.0001$) with post hoc tests identifying a statistically significant association for BG level ($P < 0.0001$), GSH ($P < 0.0001$), D-dimer ($P < 0.02$) and total cholesterol ($P < 0.0001$). Of the subjects who showed hyperglycaemia-associated progression in clinical and biochemistry status, 89% had low-level GSH and 44% had high-level D-dimer. Four individuals exhibited prediabetic status at some stage and would qualify for macrovascular disease intervention. The results of this study suggest that BG level, D-dimer, GSH and total cholesterol contribute significantly to a diabetic oxidative damage panel of markers that could assist in evidence-based pharmacological intervention with anti-aggregation and/or antioxidant agents against future CVD in diabetes.

Lippi G, Montagnana M, Salvagno GL, Guidi GC. Influence of stable, long-term treatment with phenobarbital on the activity of serum alanine aminotransferase and gamma-glutamyltransferase. *Br J Biomed Sci* **2008; 65(3): 132-5.**

Phenobarbital, a long-acting barbiturate, is generally considered to be a fairly safe and effective drug; however, hepatotoxicity is an infrequent but potentially fatal adverse effect and there is little information on the serum activity of liver enzymes in patients on stable, long-term monotherapy. The serum activity of alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) are measured along with phenobarbital as part of the routine biochemical measurement in 128 consecutive adult out-patients on stable, long-term phenobarbital treatment. The control population consists of 2468 consecutive outpatients matched for age and gender. The patients on long-term phenobarbital therapy had significantly higher serum activities of ALT (27 IU/L versus 23 IU/L, $P < 0.001$) and GGT (79 IU/L versus 24 IU /L, $P < 0.001$). The prevalence of subjects with abnormal GGT values, but not ALT, was significantly higher than that in the control population. No significant differences were observed in either the mean activity or the prevalence of abnormal values of ALT or GGT between patients with suboptimal and therapeutic concentrations of the drug. These results suggest that chronic phenobarbital therapy may be associated with a clinically significant elevation of serum GGT activity. If confirmed, a specific GGT reference range should be adopted. Moreover, in those patients presenting with high serum GGT activity, it would not be necessary to reduce the dosage, discontinue the drug or change to a different anti-epileptic medication.

Chow E, Fox N, Gama R. Effect of low serum total protein on sodium and potassium measurement by ion-selective electrodes in critically ill patients. *Br J Biomed Sci 2008; 65(3): 128-31.*

Hypoproteinaemia may lead to spuriously high electrolyte values using indirect ion-selective electrodes (ISE) compared to direct ISEs. This study evaluates the impact on electrolyte status assessment of direct compared to indirect ISE sodium and potassium measurements in samples from critically ill patients who have a high prevalence of hypoproteinaemia. Serum sodium and potassium measurements were compared using indirect and direct ISE in 190 samples received from critical care units over a three-week period. Serum sodium and potassium measurements were higher ($P < 0.0001$) using indirect ISE (140.0 +/- 5.0 and 4.5 +/- 0.6, respectively) compared to direct ISE (136.5 +/- 5.2 and 4.5 +/- 0.6, respectively). The calculated difference between indirect and direct ISE values for sodium increased as total protein concentration decreased ($Y = 7.2-0.07X$, 95% CI slope -0.1 to -0.05, $P<0.0001$, $r2 = 0.14$). Hypoproteinaemia was present in 85% of samples. Indirect ISE, compared to direct ISE, misclassified 28% of samples as pseudonormonatraemia (19%), pseudohypernatraemia (8%), pseudonormokalaemia (0.8%) and pseudohyperkalaemia (0.4%). Hypoproteinaemia is common in critically ill patients and this may lead to spuriously high indirect ISE electrolyte measurements, resulting in significant misclassification of electrolyte (particularly sodium) status. In such patients, direct ISE (as employed in point-of-care testing) offers more accurate and consistent electrolyte results than does indirect ISE (commonly used in major laboratory analysers).

Ogunwobi OO, Beales IL. Leptin stimulates the proliferation of human oesophageal adenocarcinoma cells via HB-EGF and Tgfalpha mediated transactivation of the epidermal growth factor receptor. *Br J Biomed Sci* **2008; 65(3): 121-7.**

Obesity increases the risk of developing oesophageal adenocarcinoma (OAC) as well as several other cancers. Leptin is secreted by adipocytes and serum leptin levels rise with body mass index. Leptin stimulates proliferation and inhibits apoptosis in OAC cells but the mechanisms are not fully elucidated, Transactivation of the epidermal growth factor receptor (EGFR) is an important signalling mechanism for G-protein-coupled receptors, but the relationship with leptin-type receptors has not been examined and the authors hypothesise that leptin-induced proliferation involves EGFR signalling. This study examines the effect of leptin on EGFR signalling in cultured cell lines. Leptin stimulated proliferation in four OAC lines expressing leptin receptors (OE33, OE19, BIC-1 and FLO) and this was abolished by specific EGFR inhibitors (PD153035

and AG1478). Leptin-induced proliferation was inhibited by neutralising antibodies to transforming growth factor-alpha (TGFalpha and HB-EGF) but not by anti-amphiregulin. Leptin significantly increased gene expression of HB-EGF and TGFalpha as measured by a quantitative real-time polymerase chain reaction (PCR) method but did not alter amphiregulin and EGFR gene expression. Leptin increased extracellular release of HB-EGF and TGFalpha and this was blocked by matrix metalloproteinase (MMP) inhibitors. The MMP inhibitors also abolished leptin-induced proliferation as well as leptin-induced EGFR tyrosine phosphorylation, but did not affect proliferation or EGFR activation induced by TGFalpha. The authors conclude that leptin stimulates OAC proliferation via increased gene expression of HB-EGF and TGFalpha, MMP-mediated extracellular release of HB-EGF and TGFalpha and subsequent activation of EGFR.

Brazier JS. Clostridium difficile: from obscurity to superbug. *Br J Biomed Sci* 2008; 65(3): 39-44.

According to the UK media and popular press, *Clostridium difficile* is now a fully fledged member of that notorious but ill-defined group of microorganisms portrayed to the general public as superbugs. Following the trail blazed by methicillin-resistant *Staphylococcus aureus* (MRSA), *C. difficile* has made the transition from being an obscure anaerobic bacterium, mainly of interest to specialist anaerobic microbiologists, to that of an infamous superbug responsible for outbreaks of hospital-acquired infection that commonly result in serious disease and death. This review tracks the rise in scientific knowledge and public awareness of this organism.

News from the Universities laboratory science degree programs

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Answers to the HSIG journal questionnaire

1. Haematocrit (Hct) or PCV.
2. Viscosity can be underestimated by some analysers in the presence of iron deficiency.
3. In obese patients, low measured value and high predicted normal value.
4. Absolute erythrocytosis = RCM > 125%. Apparent erythrocytosis = < 125%
5. Above 0.60 in males and 0.56 in a female.
6. Primary, secondary and idiopathic.
7. JAK2 V617F mutation.
8. Low.
9. Congenital and acquired.
10. EPO receptor mutations, high oxygen affinity haemoglobins, biphosphoglycerate mutase deficiency, oxygen sensing pathway gene mutations, PHD2 mutations and HIF-2alpha mutations.
11. Eleven.
12. By measuring the p50 in the oxygen dissociation curve. High oxygen affinity variants shift the curve to the left.
13. They may be alpha or beta chain variants, also may be stable or unstable .
14. Yes, but 20-25% do not exhibit an abnormal band.
15. von Hippel Lindau
16. A single VHL mutation, C598T, amino acid 2000 arginine changed to tryptophan. Affected individuals are homozygous and come from the Chuvash region in Russia. Mutation has since been identified in families from outside the Chuvash area (8 families from Pakistan or Bangladesh, and another cluster from Ischia Is. Italy).
17. Central hypoxia, local renal hypoxia, excess EPO production due to a pathological process(tumour), and exogenous administration.
18. Central hypoxia.
19. 10-15%
20. Those with below normal EPO levels (one third) and those with inappropriately normal or raised EPO levels (two thirds).
21. Discovery of the JAK2 mutations.
22. A raised Hb level (>0.52 male, and >0.48 female) and a JAK2 mutation.
23. A red cell mass (RCM) to determine a "true" increase in red cells.

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