Volume 58 Number 3 November 2004 ISSN 1171-0195

New Zealand Journal of Medical Laboratory Science

Official Publication of the New Zealand Institute of Medical Laboratory Science Incorporated

Basics and Beyond
Christchurch 2005
14-18 August

NZ Institute of Medical Laboratory Science
Annual Scientific Meeting

Christchurch Convention Centre

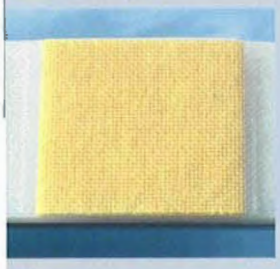
3

Roche

Diagnostics

Combur-Test® Product Line

Urine test strips for unequalled performance



Roche Diagnostics NZ Ltd
Toll Free: 0800 652 634
Email: bronwyn.sheppard@roche.com

All editorial matter including submitted papers, press releases and books for review should be sent to the Editor: Rob Siebers, Dept. of Medicine, Wellington School of Medicine, PO Box 7343, Wellington South. Phone: (04) 385 5999 Ext. 6838. FAX: (04) 389 5725. E-mail: rob@wnmeds.ac.nz. Comprehensive instructions for authors can be found in the *N Z J Med Lab Science* 2000; 54(3): 108-10 or on the website (www.nzimls.org.nz). Submit two copies of the manuscript (and on disk if possible) together with a covering letter from the corresponding author stating that the work is original, is not under consideration for publication elsewhere, that all listed authors have contributed directly to the planning, execution, analysis or to the writing of the paper, and that all cited references have been checked against the original article or appropriate data bases. Contributors and advertisers are responsible for the scientific content and views. The opinions expressed in the Journal are not necessarily those of the Editor or Council of the NZIMLS

The New Zealand Journal of Medical Laboratory Science is the official publication of the New Zealand Institute of Medical Laboratory Science (NZIMLS) who owns the copyright. No parts of this publication may be reproduced in anyway without the written permission of the NZIMLS. The Journal is published three times per year in April, August and November, printing by Centurion Print, PO Box 6344, Wellesley St., Auckland.

The Journal is abstracted by the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Index Copernicus, Excerpta Medica/EMBASE, Chemical Abstracts, and the Australian Medical Index. The journal, through its Editor, is a member of the World Association of Medical Editors (WAME).

Advertisement bookings and enquiries should be addressed to the Advertising Manager: Trish Reilly, 48 Towai St., St Heliers, Auckland 5. Phone: (09) 575 5057, Fax: (09) 575 0698.

Enquiries regarding subscription and address changes should be addressed to the Executive Officer of the NZIMLS: Fran van Til, PO Box 505, Rangiora. Phone: (03) 313 4761.
E-mail: fran@eenz.com.

New Zealand Journal of
Medical Laboratory Science
Volume 59 Number 3
November 2004
ISSN 1171-0195

Editorial

Prostate specific antigen: to screen or not to screen

Rob Siebers, FNZIMLS
Wellington School of Medicine and Health Sciences

Deaths attributable to prostate cancer in New Zealand reached 531 in 1997 (3.8% of all male deaths) and were projected to rise to 844 in 2012 (1). Serum prostate specific antigen (PSA) and digital rectal examination (DRE) are the two screening procedures used in the diagnosis of prostate cancer. There has been a rapid increase in reported prostate cancer incidence since 1991, most likely due to an increase in PSA testing with the majority of GPs screening men who ask about screening for prostate cancer (2).

In 1996 the National Advisory Committee on Health and Disability (the National Health Committee, NHC), after review, advised against population screening for prostate cancer, but advised that it should be kept under review. In 2004 the NHC, using the report by the New Zealand Guidelines Group Prostate Screening Advisory Group (3) and the NHCs screening assessment criteria (4) stated that *'the National Health Committee does not currently support population-based screening for prostate cancer or opportunistic screening using PSA or DRE for asymptomatic men in New Zealand'* (5).

All members of the New Zealand Guidelines Group Prostate Screening Advisory Group agreed that there was currently no evidence to support a nation-wide screening programme, however, there was a wide divergence of opinion within the Group on the issue of opportunistic screening. Some believed that men should not be offered PSA screening due to lack of beneficial evidence and potential for harm, while others supported PSA testing, provided men were informed on the risks and potential benefits of the test.

In this issue of the Journal, Professors Delahunt and Nacey and Dr Lamb from the Wellington School of Medicine and Health Sciences put forward their views on the diagnosis of prostate cancer (6). Professor Delahunt was a member of the New Zealand Guidelines Group Prostate Screening Advisory Group. Their main argument is that screening for prostatic cancer is beneficial for selected men, particularly those with a positive family history were there is up to an eleven-fold risk of developing prostatic cancer.

In support of their recommendation not to support population-based screening nor opportunistic screening, the NHC argue that prostate cancer usually develops slowly and that many men with prostate cancer do not die from it. Also, that the side effects of treatment results in a poorer quality of life in men with a positive PSA (> 4ng/ml). Delahunt and colleagues argue that this does not recognise that simultaneous DRA and % free-PSA greatly increases detection of prostate cancer in men who have a total PSA of < 4ng/ml on one occasion. They also argue that PSA velocity (2ng/ml/year) is virtually diagnostic (7).

Recent studies published in 2004 may be of use in determining whether screening for prostate cancer is of use. Roobol and colleagues cast doubt on the use of PSA velocity in those with a PSA level of < 4ng/ml to detect prostate cancer (8). They conclude that a large proportion of prostate cancers that are potentially curable can be found at PSA levels of < 4ng/ml but that PSA velocity is not a useful screening tool for the detection of prostate cancer. D'amico and colleagues conclude that men with a PSA velocity > 2ng/ml/year before prostate cancer diagnosis have a relatively high death rate despite undergoing radical prostatectomy (9). Thompson and colleagues showed that biopsy-detected prostate cancer, including high grade cancers, was not uncommon in men with PSA levels of < 4ng/ml (10). Raaijmakers and colleagues showed that % free PSA was predictive of tumour aggressiveness and could be used to select treatment options (11).

Thus, these, and other studies (12) show that various forms of PSA screening may be of use in detection of prostate cancer. None of the various PSA options (total PSA, % free PSA, PSA velocity) are without its limitations. Also, further research is required to determine whether other molecular forms of PSA improve discrimination between benign and malignant prostatic disease (13). What is needed is a clear algorithm, continuously updated in light of new findings, and cooperation between surgeons, clinicians, epidemiologists, and medical laboratory specialists, to determine what is the best screening strategy for selected men to detect the 3rd most common cause of male cancer death in New Zealand.

References

1. Cancer in New Zealand. Trends and Projections. Public Health Intelligence Occasional Bulletin No. 15. Ministry of Health, Wellington, 2002: pp 338.

2. Durham J, Low M, McLeod D. Screening for Prostate Cancer: A Survey of New Zealand General Practitioners. National Guidelines Group, Wellington, 2002: 1-43.

3. Prostate Cancer Screening in New Zealand. Appendix 2. Population-Based Screening for Prostate Cancer and Testing of Asymptomatic Men in New Zealand. National Health Committee, Wellington, 2004: 33-52.

4. Screening to Improve Health in New Zealand. National Health Committee, Wellington, 2003: 7.

5. Prostate Cancer Screening in New Zealand. Report to the Minister of Health. National Health Committee, Wellington, 2004: pp 52.

6. Delahunt B, Lamb DS, Nacey JN. The diagnosis and treatment of prostate cancer: will commonsense prevail? *N Z J Med Lab Sci* 2004; 58: 00-00.

7. Smith DS, Catalona WJ. Rate of change in serum prostate specific antigen levels as a method for prostate cancer detection. *J Urol* 1994; 152: 1163-7.

8. Roobol MJ, Kranse R, de Koning HJ, Schroder FH. Prostate-specific antigen velocity at low prostate-specific antigen levels as screening tool for prostate cancer: results of second screening round of ERSPC (ROTTERDAM). *Urology* 2004; 63: 309-13; discussion 313-5.

9. D'amico AV, Chen MH, Roehl KA, Catalona WJ. Preoperative PSA velocity and the risks of death from prostate cancer after radical prostatectomy. *N Engl J Med* 2004; 351: 125-35.

10. Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, et al. Prevalence of prostate cancer with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med* 2004; 350: 2239-46.

11. Raaijmakers R, Blijenberg BG, Finlay JA, Rittenhouse HG, Wildhagen MF, Roobol MJ, et al. Prostate cancer detection in the prostate specific antigen range of 2.0 to 3.9 ng/ml: value of percent free prostate specific antigen on tumor detection and tumor aggressiveness. *J Urol* 2004; 171: 2245-9.

12. Finne P, Finne R, Bangma C, Hugosson J, Hakama M, Auvinen A, et al. Algorithms based on prostate-specific antigen (PSA), free PSA, digital rectal examination and prostate volume reduce false-positive PSA results in prostate cancer screening. *Int J Cancer* 2004; 111: 310-5.

13. Gretzer MB, Partin AW. PSA markers in prostate cancer detection. *Urol Clin North Am* 2003; 30: 677-86

TH Pullar Memorial Address
Outside, looking in

Rob Siebers, MIBiol, FNZIC, FNZIMLS
Wellington School of Medicine and Health Sciences, Wellington

Mr President, distinguished guests, colleagues and friends. I feel honoured to have been invited to give the TH Pullar Memorial Address at this years conference. I thought long and hard what a suitable topic might be. I looked through previous addresses given by many prominent members of our profession, such as John Case, Des Phillips (twice), Jan Parker, Marilyn Eales, Ron Mackenzie, Barry Edwards, just to name a few. Should I talk about education, a subject dear to Dr Pullar's heart. However, apart from training medical laboratory scientists at the bench and some teaching at the Polytechnic to senior students many years ago, I have not been directly involved in teaching recently. Or should I talk about the Journal, of which I have been Editor the last ten years. However, this was adequately covered by Bob Allan, previous Editor of the Journal, in his TH Pullar Memorial Address.

I have been away from the bench since 1983, 21 years ago. Therefore I feel that I cannot directly talk about various issues that affect our profession today, certainly not at the bench level. However, I have stayed in touch with the profession during those years by, among other, regular attendance at various medical laboratory science meetings, being Editor, and on the Board of Studies of the Otago BMLSc degree course. Thus, feeling somewhat of an outsider, and given the theme of this year's conference being 'Windows in Waikato', I thought I would entitle my talk 'Outside, looking in'. I will attempt to look through my crystal ball and try and predict what may lie ahead for our profession over the next five years. In order that in the future I would not solely be held responsible for getting it totally wrong, over the last six weeks I have asked various friends in the profession, who are at the coal face, to try and predict what the near future holds for us and what the burning issues are. However, they will remain anonymous, they know who they are. But before embarking on this navel gazing exercise, I want to briefly answer the question of who Dr Pullar was and why the Institute honours his name each year at the Annual Scientific Meeting.

Dr Toss Pullar was born in Auckland in 1907. His father was a GP and returned with his family to Scotland. Dr Pullar attended Glasgow and Sheffield Universities, the latter where he graduated MB ChB (Hons) in 1929. After various positions and training in pathology in the UK, he returned to New Zealand in 1937 to take up the position of Pathologist at Palmerston North Hospital, a position he held for the next 25 years. Dr Pullar was a champion and great friend of New Zealand medical laboratory technologists, as we were known then, during those years. He build up professional laboratory standards throughout the country, was involved in the formation of the Medical Laboratory Technologists Board, and helped draft conditions of employment in medical laboratories throughout New Zealand. His greatest contribution to our profession at that time was undoubtedly through his involvement in the training of medical laboratory technologists. He prepared syllabi for the intermediate exams and was for many years examiner. Even after moving to Tauranga in 1973 due to deteriorating health, he continued to visit laboratories throughout the country setting up and supervising exams and introducing new educational training schemes. Dr Pullar was a livelong friend, teacher and champion of our profession and it is fitting that to this day the NZIMLS continues to recognise him through the TH Pullar Memorial Address.

One of the main issues affecting our profession over the next five years is an increasing shortage of qualified and experienced health professionals, including laboratory staff. There is a greying of this work force in New Zealand. The Ministry of Health in 2000 put the median age of health professionals in the mid-forties. After many years of lobbying by the profession we finally achieved in 1991 a university-based qualification with the first group of graduates from Massey and Otago Universities emerging 10 years ago. The move from an on-the-job training scheme for medical laboratory technologists, as we were then known, to a university-based degree programme, together with the introduction of user pays university fees, means that the total cost of medical laboratory science education has increased dramatically. Under the previous on-the-job training, employers absorbed this cost of training. Now, it is totally absorbed by the student, leaving them with a substantial student loan to repay when they enter the work force as a fully fledged medical laboratory scientist. However, the employers point of view is that, as they achieve similar levels of productivity after qualifying, pay rates have not changed significantly or proportionally. Thus, it is not surprising, given the current levels of remuneration for recently qualified graduates, together with their substantial student loan debts, that they look for greener pastures overseas where New Zealand trained medical laboratory scientists are, and have been, highly regarded. We must also not forget that universities will urge high achieving students to pursue post graduate qualifications to then go into research or medical training. Anecdotally, about 10% of medical laboratory science graduates each year are thus 'lost' to the profession.

Key issues for the medical laboratory scientists group in the 2001 New Zealand Health Workforce Report were:

- Growing evidence of shortages in both the number of trainees and practicing medical laboratory scientists. The New Zealand Institute of Medical Laboratory Science estimated then that, due to difficulties in filling vacancies, there was a need to produce at least 120 graduates each year. In 2001 the three universities, AUT, Massey, and Otago produced about 70 graduates, a figure likely to be similar in 2004. There was also concerns regarding English language skills in a significant number of students, making it difficult for them to find employment after graduation.
- In 2001 there was a perceived low morale that was partially due to negative publicity for high profile public enquiries, such as the Gisborne affair.
- Remuneration - there was a perceived lack of pay relatively with other allied health professionals within New Zealand and with medical laboratory scientists in other countries.

Looking into my crystal ball and helped by comments from colleagues in the laboratory work place, I predict this situation to continue, and perhaps worsen over the next five years. Currently there is another round of restructuring coming up, with reviews underway by a number of District Health Boards This will undoubtedly lead to a further reduction in the work force with limited opportunities for medical laboratory scientists within the industry. More work will have to go through the analysers by fewer medical laboratory scientists leading to problems when things go wrong, and the routine medical laboratory becoming more like a factory.

The new HPCA bill will, I believe, have a major impact in the work place over the next five years. From the middle of September 2004 the Medical Laboratory Scientists Board will maintain a register of laboratory health professionals, each of whom will have an approved scope of practice appropriate to the laboratory health professionalís qualification and laboratory experience. There will be two scopes of practice approved by the Board, namely medical laboratory scientist and medical laboratory technician, both titles to be protected. All current registered medical laboratory staff, whether they qualified with a Batchelor of Medical Laboratory Science, or a New Zealand Diploma in Medical Laboratory Technology (or its predecessor qualification), will retain registration under the new regulations. From mid September the Batchelor of Medical Laboratory Science degree from a New Zealand university will be the main qualification for a medical laboratory scientist's scope of practice. Additionally, for those without a Batchelor of Medical Laboratory Science degree, but with a suitable post-graduate qualification combined with relevant and specialised medical laboratory experience will be considered by the Board for registration as a medical laboratory scientist. At present there are a number of medical laboratory health professionals without a Batchelor of Medical Laboratory Science pursuing a post-graduate Diploma in Science for them to be registered. The qualification for the medical laboratory technicianís scope of practice will primarily be a QTA or QTP certification from the Institute. Additionally, a BSc or Certificate in Science with a minimum of 12 months full-time relevant or equivalent medical laboratory experience, will be considered by the Board for registration as a medical laboratory technician.

So, what impact will these new regulations have on the profession over the next five years? The main one, I believe, is the requirement of medical laboratory scientists to show continuing competence and professional development, as part of the requirements to hold an annual practicing certificate (APC). To hold a current APC, the Board has stated that the medical laboratory scientist will be required to participate in, and demonstrate satisfactory performance, in an approved competency programme. Currently, the Institute's CPD programme has been accredited by the Board for this purpose.

Briefly, the medical laboratory scientist will have to accumulate approximately 100 points per year, with a minimum of 300 points required for any three year period. There is a compulsory section of 60 points each year based on the annual training document review, conducted as part of the in-laboratory training requirements of IANZ accreditation. Thus, an additional 140 points are required over the three year period from the other 17 categories of activity, such as continuing education, teaching, and professional affairs. For the full-time employed medical laboratory scientist acquiring these additional 140 points over three years (about 50/year) should not be to difficult. For example, one full day attendance at this conference, or a SIG meeting, will give you 16 points, and attendance at a half-day work shop, eight points. Additionally, if you are giving an oral presentation at this meeting you will get 20 points. Another activity to obtain points is to publish a paper (preferably in the Institute's Journal) for which you can obtain a maximum of 20 points.

There is a perceived problem of obtaining the additional 140 points for part-time and night-time medical laboratory staff who may, due to restraints such as family commitments, find it more difficult to attend approved scientific meetings. However, there are other approved activities where they can obtain the required number of points, such as structured reading, self assessment programmes and web based learning. All eligible medical laboratory scientists must show continuing competence to hold an ACP. This is perhaps more pertinent for the part-time or night-time medical laboratory scientist.

The CPD programme of the Institute evolved from the MOLS programme. Many refinements and fine tuning have ensured over the years to what, I believe, is a very good programme that will serve the profession well. Although it is currently the Board's accredited programme for competence and professional development, that does not mean it will always be so. Other professional groups and organizations may, and in my opinion will, in the future present programmes to the Board which they believe will serve our profession better than the current CPD programme. It is up to the Institute and to you as purchaser of this programme, to ensure that the CPD programme will continue to fulfil its purpose. Therefore, problems with the programme, or suggestions on how to improve it will need to be communicated by the profession to the Institute.

Over the next five years I believe the medical laboratory health professional will become more specialised. In the pre-degree phase of our training we did a common core component before specialising in one or two specific disciplines of medical laboratory science. With the advent of the degree in medical laboratory science a more generally trained medical laboratory scientist emerged. Now, with the imminent start of an approved scope of practice we will see a shift back to specialisation. There will always be a need for the 'all-rounder' in the medical laboratory, especially in the smaller centres. But in the bigger centres more specialist work will be done by medical laboratory scientists with post-graduate qualifications, such as MSc, PhD and may I suggest, Fellowship of the Institute. Medical laboratory scientists going into management will most likely need appropriate qualifications, such as BCom or MBA. As for the medical laboratory scientist going into the teaching programmes of the three universities, a post-graduate qualification is mandatory, especially in the current PBRF (performance based research funding) era and requirements for accreditation of the Batchelor of Medical Laboratory Science degree programmes.

Medical laboratory health professionals will have to be flexible if they want to move forward over the next five years. Technology is continuously evolving and will drive the changes. We must keep up with these changes through continuous education and professional development. A key area, I believe, will be molecular diagnostics. This discipline will impact on all areas of medical laboratory science, be it clinical biochemistry, haematology, transfusion medicine, immunology, microbiology or histology. We will also see significant changes in the afore mentioned disciplines of medical laboratory science. For instance, there will be a need for a strong microbiology laboratory service what with the continuing emergence of antibiotic-resistant pathogens, and the rapid emergence and spread of epidemics such as SARS. There will also be a continuous development of new tests to aid in patient diagnosis. Recent examples of this has been the establishment of Troponin as a marker of cardiac tissue damage, and the development of BNP as a test to detect heart failure. I am sure there will be more specific markers being developed and introduced in the medical laboratory over the next five years.

Thus, with all the rapid developments and forever changing scenes in the medical laboratory over the preceding five years and no doubt continuing this march over the next five years and beyond, it is imperative that the medical laboratory health professional is able to adapt to those changes. I believe that the specialised university training our graduates receive prepares them well to be able to adapt to these changes. May I also make a plea for us to call ourselves medical laboratory scientists, not technologists. Our professional body has for many years been named the New Zealand Institute of Medical Laboratory Science, and this year sees the Medical Laboratory Technologists Board change its name to reflect that we are scientists, not technologists.

I wish to thank this yearís conference organising committee for putting my name forward and Council for inviting me to give the TH

Pullar Memorial Address. Furthermore, to my colleagues and friends for their valuable comments and insights into where they believe medical laboratory science is heading over the next five years. If Dr Pullar was around today I am sure he would agree that the education of the medical laboratory scientists is appropriate and of a high standard to meet the requirements of the medical laboratory today and beyond. He would also agree that to be able to adapt to the inevitable changes that will happen in the future we must continue with our professional development. Thank you for listening to an 'outsider', looking in.

Address for correspondence: Rob Siebers, Department of Medicine, Wellington School of Medicine and Health Sciences, PO Box 7343, Wellington South. Email: rob@wnmeds.ac.nz.

Neonatal alloimmune thrombocytopenia. A review

Susan Evans, FNZIMLS,Staff Medical Laboratory Scientist, Blood Bank Laboratory, New Zealand Blood Services, Wellington

Abstract

Neonatal (feto-maternal) alloimmune thrombocytopenia (NAIT or FMAIT) is due to maternal alloimmunisation to fetal platelet antigens. Maternal IgG alloantibodies cross the placenta, causing destruction of fetal platelets. The clinical manifestations in the newborn may range from minor bruising of the skin to intracranial haemorrhage (ICH) to intrauterine death in severe cases.

Human platelet antigens (HPA) are expressed on platelet membrane glycoproteins, and are biallelic amino acid polymorphisms based on nucleotide substitutions. The distribution of platelet alloantigens varies between different ethnic groups. The platelet-specific alloantibodies which have the most clinical significance in Caucasian populations are anti-HPA-1a and anti-HPA-5b specificities. At present, there is no screening programme to identify those at risk.

With anti-human leukocyte antigen (HLA) alloantibodies frequently formed during pregnancy and HLA class I antigens expressed on the platelet surface, it is not clear whether HLA antibodies cause NAIT. HLA class II molecules may have a role in B cell response to HPA alloantigens, and may be HLA class II restricted, with 98% of anti-HPA-1a alloantibody responders in one study possessing the HLA-DRB3*0101 allele.

The diagnosis of NAIT is initially made on clinical grounds after exclusion of other causes of thrombocytopenia. Identification of platelet-specific alloantibodies can be carried out using a number of different techniques. However, improvements in assay performance are required in those cases of NAIT where no antibody is identified. Phenotyping or genotyping a mother for platelet-specific antigens is essential for confirming a platelet antibody's specificity. DNA-based methods for HPA typing are becoming more widely and readily available.

Due to the high risk of recurrence of NAIT in subsequent pregnancies, there is an urgent need for a reliable and non-invasive test to predict likely severity of FMAIT in at risk infants. Currently there is no laboratory test procedure available.

This review examines the causes, treatment and laboratory diagnosis of neonatal alloimmune thrombocytopenia as reported in the scientific literature, and was researched and written to increase awareness and a better understanding of the potential seriousness of the condition. Data sources were primarily obtained by a manual search of mainly five English language journals.

Key words: neonatal, fetal, alloimmune, thrombocytopenia, alloantigen, alloantibody, IgG, NAIT, FMAIT, HPA, HLA, ICH

Introduction

Immunisation to platelet-specific alloantigens is the primary cause of neonatal (feto-maternal) alloimmune thrombocytopenia (NAIT, FMAIT). Alloantibodies are formed as a consequence of maternal immunisation to fetal platelet-specific alloantigens of paternal origin. The maternal IgG alloantibodies cross the placento-fetal barrier via active transport in syncytiotrophoblast and endothelial cells as early as the fourteenth week of gestation (1). This results in immune mediated platelet destruction in an otherwise healthy newborn. Active transport is mediated by the neonatal Fc receptor (FcRn) which binds IgG with a pH-dependent affinity.

Early reports on NAIT have quoted an incidence ranging between 1 in 2500-5000 live births per year (2), with the affected neonates suffering clinically recognised symptoms. With greater awareness of the condition and improved serological techniques for platelet antibody identification, estimates of the incidence have increased from 1 in 1000-2000 (3), and then to 1 in 800-1000 (4-6). Recognition of platelet alloimmunisation without severe thrombocytopenia can now be used in the risk assessment of subsequent siblings providing a better prognosis than previously.

The pathophysiology of NAIT is parallel to that of Rh haemolytic disease of the newborn (HDN) (7), except that it usually affects the first baby, with about 40% (3) to 60% of NAITs occurring during the first pregnancy (8,9). It recurs in over 90-95% of subsequent pregnancies with incompatible fetuses at least as severely affected as the previous (7,10). However, alloimmunisation does not always result in NAIT (4). Some neonates may be asymptomatic, with thrombocytopenia discovered incidentally (3,7).

Clinical features

The condition develops in *utero*, with the fetus mildly to severely affected. The most serious complication is ICH in 10-20% of cases. Of these, up to 10% are fatal and 20% develop long-term neurological impairment (3). Severe thrombocytopenia (<30 x 10^9/L) can occur during early pregnancy (11), and in *utero* ICH has been observed before 20 weeks of gestation (7).

Diagnosis

Usual presentation is that of a healthy mother with a normal platelet count having given birth to a full-term neonate with wide-spread purpura present at birth or developing shortly afterwards (7). The diagnosis of NAIT is made initially on clinical grounds, after exclusion of other causes of thrombocytopenia. A history of thrombocytopenia in previous siblings is a strong indication for the diagnosis (7). Confirmation requires laboratory testing to detect a maternal alloantibody with specificity against the baby's platelets. A parental HPA-1a mismatch (mother HPA-1a negative, father HPA-1a positive) is the commonest cause of NAIT in a thrombocytopenic infant, even when anti-HPA-1a antibody is not detected in maternal serum. In contrast, in the other HPA systems where there is parental incompatibility but no corresponding anti-HPA alloantibody in the maternal serum, the diagnosis of NAIT is less likely (4).

Antenatal management of subsequent pregnancies where an infant has been affected by NAIT will be determined by whether the father is homozygous or heterozygous for the relevant platelet antigen. For mothers with the HPA-1b1b genotype (i.e. HPA-1a negative), half of subsequent fetuses who have an HPA-1a1b father (genotype frequency 28%) will not be affected. Determining the father's zygosity is especially important in counselling a couple.

Prenatal diagnosis may be suspected by ultrasound showing intracranial cyst or ventriculomegaly. Confirmation of the diagnosis and severity of FMAIT in *utero* depends on percutaneous umbilical blood sampling using ultrasound guidance. This invasive procedure carries a mortality risk of greater than 1% in a thrombocytopenic fetus (10), which depends on the skill of the operator. Fetal molecular

genotyping is possible for the HPA polymorphisms when the father is heterozygous for the implicated antigen, which may help to predict the risk of FMAIT.

Differential diagnosis of neonatal thrombocytopenia

The platelet count of an 18 week fetus and a newborn is similar to the adult range of 150 - 400 x 10^9/L (12). Neonatal thrombocytopenia is defined as a platelet count <150 (10^9/L for term infants and <100 (10^9/L for low birth weight infants (13).

The frequency of fetal/neonatal thrombocytopenia in all newborns has been estimated at 1-4% (4,7,10). An immune mechanism was likely to be the cause in 0.3% of cases overall, due to either NAIT or maternal autoimmune thrombocytopenic purpura (AITP), where maternal IgG platelet-reactive autoantibodies can recognise fetal as well as maternal platelet antigens (4,14). Patients with AITP generally produce autoantibody against one or more platelet glycoproteins or portions of glycoproteins rather than against the specific platelet antigen. In many cases, antibody may be present only on the mother's platelets and not in the serum, known as platelet-associated IgG (PAIgG). The maternal thrombocytopenia may be idiopathic, drug related (quinine, sulphur drugs and heparin are the most frequently incriminated) or HIV related (14). Women with severe AITP during pregnancy were found to have a high risk of severe fetal thrombocytopenia, particularly if they had previously had a splenectomy (15). A very low risk of severe fetal thrombocytopenia was found in women who had not had a splenectomy or severe thrombocytopenia during pregnancy. NAIT may also be present simultaneously with maternal AITP (4,7).

Thrombocytopenia is reported to affect 20-40% of infants in neonatal intensive care units (4). Viral infectious diseases known as TORCH (toxoplasma, rubella, cytomegalovirus (CMV), herpes) may interfere with megakaryocyte maturation in the fetus (16), with CMV causing the most severe thrombocytopenia among this group (10). Sepsis, chromosomal abnormalities, HDN, maternal pre-eclampsia, and gestational thrombocytopenia are among other known causes of neonatal thrombocytopenia (10,17).

Pathogenesis

Circulating cells such as platelets are usually destroyed *in vivo* by a combination of humoral and cellular mechanisms. The platelets become sensitised with antibody, with or without the activation of complement. mononuclear phagocytic cells (MPC) (e.g., splenic macrophages and Kupffer cells in the liver) possess Fcγ receptors for IgG1, IgG3 and IgG2 subclasses. Once attached to the MPC, the cell may be destroyed by antibody-dependent cellular cytotoxicity and/or phagocytosis.

Platelets may also be destroyed following complement activation (18). IgM, IgG1 and IgG3 can activate complement to form the membrane attack complex. Platelet membrane lesions develop and the cell is destroyed intravascularly. IgG2 and IgA rarely cause intravascular destruction of circulating cells.

The effector cells responsible for the cellular mechanism are macrophages and lymphocytes (cytotoxic T-cells and natural killer (NK) cells). Cytotoxic T-cells recognise foreign antigens, and together with HLA class II molecules on antigen-presenting cells (APC), are stimulated into blast cells and proliferation. Activated helper T-cells release cytokines which attract macrophages, and also help cytotoxic T-cell precursors to become killer cells. NK cells are large granular lymphocytes, which recognise determinants on a foreign cell and release the contents of the NK cell's granules. One of the components is perforin, which is similar to C9 in the complement system. Perforin can create a transmembrane pore leading to cell destruction. Garratty believes there is a possibility that this mechanism may be responsible for platelet destruction when no antibodies are detected (18).

HLA molecules are receptors which capture peptide fragments of antigen that are displayed (presented) on the cell surface where they can be recognised by appropriate T cells. Class I molecules are expressed on the cell membranes of all nucleated cells, whereas class II molecules are confined to B lymphocytes, dendritic cells and some endothelial cells. HLA molecules play a major role in regulating the immune response by controlling the activation state of T and NK cells (19).

Molecular genetics of platelet-specific alloantigens

The existence of platelet-specific alloantigens has been recognised since the early 1950's. The first recognised and clinically most important platelet alloantigen, Zw^a (HPA-1a), was observed by van Loghem et al (1959) in a patient who developed post-transfusion purpura (20).

To date, 24 platelet-specific alloantigens have been defined by immune sera, of which 12 are grouped in six biallelic systems (HPA-1, -2, -3, -4, -5, -15), as shown in column 1 of Table 1. For the remaining 12, alloantibodies against low frequency antigens have been described. Two of these are without an HPA assignment until the genetic basis has been determined (Table 2). The high-frequency allele of a system is designated with the letter "a" and the low-frequency allele with the letter "b". The letter "w" (workshop) indicates where antibodies against only one allele have been identified. In these instances, even though the single point mutations in the wild-type DNA are known, a space has been left for the antithetical antigens to be reported later when they are observed.

The second column (Table 1) lists the original name of the alloantigen, designated by the first two or three letters of the surname of the patient who produced the first alloantibody, followed by ^{a} or ^{b}, depending on frequency in the population.

The platelet-specific alloantigens are located on five platelet membrane glycoprotein structures, which play an important role in platelet activation. The majority of polymorphisms have been localized to glycoprotein IIIa (GPIIIa), which together with GPIIb forms the receptor for fibrinogen (2). Other polymorphisms are located on GPIIb, GPIa (forming the receptor for collagen), and GPIb((part of the receptor for von Willebrand factor).

The glycoproteins expressing each of the various alleles are listed in the third column (Table 1). Currently, eight different GPIIIa alleles, three variants of GPIa and GPIIb and two of the $GPIb_\alpha$ and $GPIb_\beta$ subunits have been found in the human gene pool (21).

Table 1. Human platelet antigens

Antigen	Synonym	Glycoprotein location	Nucleotide substitution	Aminoacid substitution
HPA-1a	Zw^a, PI^{A1}	GPIIIa	T_{176}	Leu_{33}
HPA-1b	Zw^b, PI^{A2}		C_{176}	Pro_{33}
HPA-2a	Ko^b	GPIbα	C_{482}	$Thr1_{45}$
HPA-2b	Ko^a, Sib^a		T_{482}	Met_{145}
HPA-3a	Bak^a, Lek^a	GPIIb	T_{2621}	Ile_{843}
HPA-3b	Bak^b		G_{2621}	Ser_{843}
HPA-4a	Yuk^b, Pen^a	GPIIIa	G_{506}	Arg_{143}

HPA-4b	Yuk^a, Pen^b		A_{506}	Gln_{143}
HPA-5a	Br^b, Zav^b	GPIa	G_{1600}	Glu_{505}
HPA-5b	Br^a, Zav^a, Hc^a		A_{1600}	Lys_{505}
HPA-6bw	Ca^a, Tu^a	GPIIIa	A_{1544}	Gln_{489}
			G_{1544}	Arg_{489}
HPA-7bw	Mo^a	GPIIIa	G_{1297}	Ala_{407}
			C_{1297}	Pro_{407}
HPA-8bw	Sr^a	GPIIIa	T_{1984}	Cys_{636}
			C_{1984}	Arg_{636}
HPA-9bw	Max^a	GPIIb	A_{2602}	Met_{837}
			G_{2602}	Val_{837}
HPA-10bw	La^a	GPIIIa	A_{263}	Gln_{62}
			G_{263}	Arg_{62}
HPA-11bw	Gro^a	GPIIIa	A_{1976}	His_{633}
			G_{1976}	Arg_{633}
HPA-12bw	Iy^a	$GPIb_\beta$	A_{119}	Glu_{15}
			G_{119}	Gly_{15}
HPA-13bw	Sit^a	GPIa	T_{2483}	Met_{799}
			C_{2483}	Thr_{79}
HPA-14bw	Oe^a	GPIIIa	$_{1909\_1911del}AAG$	Lys_{611del}
HPA-15a	Gov^b	CD109	C_{2108}	Ser_{682}
HPA-15b	Gov^a		A_{2108}	Tyr_{682}
HPA-16bw	Duv^a	GPIIIa	T_{497}	Ile_{140}
			C_{497}	Thr_{140}

Table 2. Platelet antigens without HPA assignment

Antigen	Glycoprotein
Va^a	GPIIb/IIIa
Mou^a	unknown

Since the late 1980's, molecular biology has been used to determine the genetic basis for the alloantigenic epitopes of the platelet alloantigens. The messenger RNA (mRNA) in platelets can be converted to a DNA copy of each mRNA molecule (cDNA) by reverse transcription. Sequencing is used to detect nucleic acid polymorphisms associated with the different alloantigens (2). So far all known platelet alloantigen systems are based on single nucleotide substitution leading to single amino acid polymorphism in the various membrane glycoproteins (23). The fourth and fifth columns (Table 1) include details of the amino acid substitution generally caused by a single nucleotide polymorphism in the gene encoding the relevant membrane glycoprotein for each HPA system. For example, HPA-1a and 1b alleles are due to a single amino acid substitution of leucine to proline at amino acid residue 33 of GPIIIa caused by a thymine (T) to cytosine (C) base pair substitution at position 176 of the GPIIIa gene.

The antigen Nak is not the result of an amino acid substitution. Nak^a epitope appears to be located on GPIV and Nak^a negative individuals have a deficiency of platelet GPIV, thus Nak^a is an isoantigen (5).

Many platelet alloantigens have subsequently been found on other cells and tissues as well. Platelet alloantigens residing on GPIIIa have been detected on endothelial cells (24), smooth muscle cells, and fibroblasts (25). Therefore, anti-HPA-1a antibodies may contribute to the characteristic purpura of NAIT by causing vascular damage (26). Antigens residing on the GPIa subunit have been found on activated T lymphocytes and endothelial cells (27). In contrast, alloantigens localized on GPIIb, $GPIb\alpha$ and $GPIb\beta$ subunits appear to be unique to platelets (21).

The glycoprotein IIIa is present as early as 16 weeks' gestation and expresses HPA-1a alloantigen in normal amounts (12), so alloimmunisation can lead to fetal thrombocytopenia as early as 20 weeks gestation. In one case a platelet count of 15 (10^9/L was found in a 32 week old fetus (28).

Platelet alloantigens shared with other blood cells and tissues are the glycoconjugates of the blood group ABO, Lewis, I and P systems and highly polymorphic HLA class I glycoprotein molecules (5).

Epitopes of platelet-specific alloantibodies

The presence of an antibody binding site is critically dependent on the three dimensional structure of the platelet glycoprotein. The HPA-1 epitope has been found to be dependent on the presence of intact disulfide bonds, as chemical reduction results in loss of both of the HPA-1 alloantigens (29). Furthermore, some HPA-1a antibodies bind exclusively to the amino-terminal domain of GPIIIa (66 residues) and others recognise combinatorial epitopes comprising amino-terminal and cysteine-rich domains of GPIIIa (21).

In addition, some epitopes require posttranslational modifications. Unlike the HPA-1 alloepitope, the HPA-3 epitopes depend on carbohydrate modification for efficient exposure of both the HPA-3a and -3b alloepitopes (29).

Platelet-specific alloantibodies

The most common antibody (78-90%) and most clinically significant cause of NAIT in the Caucasian population is against the HPA-1a epitope on GPIIIa (3,5,14). Between 1.6% (6) and 4.6% (9) of mothers are HPA-1a negative. Around 6-12% of these women develop anti-HPA-1a alloantibodies during pregnancy (6,30), and approximately 2% of their infants develop severe thrombocytopenia (9). The frequency of alloimmunisation against the HPA-1a antigen in pregnancy is approximately 1 in 1000 in the Caucasian population (6), despite a higher number of feto-maternal HPA-1a mismatches, from 1 in 50 (6,31) to 1 in 100-200 deliveries (32).

Paradoxically, anti-HPA-1b immunisation, in the more numerous (73%) homozygous HPA-1a women, appears extremely rare (<1/540,000) (33). This differs from that observed in patients with platelet transfusion refractoriness where anti-HPA-1b accounts for 55% of the platelet-specific alloantibodies identified (23). The difference strongly suggests that, although HPA incompatibility is a pre-requisite, there are other factors involved in determining whether an immune response takes place. These are discussed in the section on NAIT and class II associations.

Incompatibility within the HPA-3 system would be expected to dominate alloimmune thrombocytopenia, with the HPA-3a antigen having a frequency of 81% and HPA-3b 70% in the Caucasian population, but only a few cases of NAIT due to anti-HPA-3a (3,34,35) or anti-HPA-3b (36) have been reported.

The most common pregnancy-induced platelet-specific antibody is anti-HPA-5b. Reported cases range from 5-15% (14) to 19-26% (3,37), but anti-HPA-5b rarely causes severe NAIT (3,32,37). This could be due to the antigen density of the HPA-5 system being lower than HPA-1 because of the fewer number of GPIa molecules (approximately 2000 binding sites) on the platelet surface compared to GPIIIa (20,000 to 50,000) (38,39). Most neonates with NAIT due to anti-HPA-5b antibodies have been delivered by multiparous women (32,37).

There have also been reports that no maternal alloantibodies are detectable in 20% of cases of NAIT (1). Occasionally, in some women, antibodies present early in the pregnancy later become undetectable (transient antibodies) until the postnatal period (30), and in others, detectable anti-HPA-1a antibodies are not developed until the post-partum period (6), sometimes weeks or months later. Systematic follow-up of subsequent pregnancies should help to answer the question as to whether neonatal thrombocytopenia is related to fetomaternal alloimmunisation in the absence of detectable alloantibodies in the maternal serum.

HLA antibodies

HLA antibodies are common in pregnant women, being detected in 1-5% of primiparous women and 30-70% of multiparous women (8), alone or in combination with HPA-antibodies (6). The role of maternal HLA antibodies in NAIT has been controversial: IgG HLA antibodies can cross the placenta and potentially interact with fetal platelets which express HLA class I antigens. Since HLA antigens are widely distributed on cells other than platelets, including placental tissues, these sites compete for binding to the HLA antibody (8). Antibodies that cross this barrier may also be neutralised by the soluble HLA molecules in the fetal circulation.

Engelfriet and Reesink have hypothesised that HLA antibodies protect against other detrimental maternal antibodies by inhibiting the Fc receptors (FcR) on fetal cells (40). There have, however, been several convincing reports of anti-HLA antibodies associated with NAIT, especially in low birth-weight infants (6,8) although in the majority of these cases, thrombocytopenia was seen in association with neutropenia (41).

Population distribution of HPA antigens

The frequency distribution of platelet alloantigens varies in different ethnic groups (Table 3), with the result that anti-HPA-1a is primarily found in the Caucasian population.

Anti-HPA-5b is the most common pregnancy-induced platelet-specific alloantibody in Asian populations (42), and anti-HPA-4b is a significant cause of NAIT in Japan (5,42).

GPIV deficiency, and therefore Naka negative, which occurs in approximately 3% of the Japanese population (43), is rare in Caucasians (5). Anti-Naka has recently been reported as a cause of NAIT (44).

Table 3. Human platelet alloantigen phenotype frequencies (5)

Antigens	Caucasians*	Maori/ Polynesian†	Koreans*	Blacks*	Japanese*	Indonesians*	Chinese*
HPA-1a	97.9	>99.9	>99.9	99.6	>99.9	>99.4	>99.9
HPA-1b	28.6	n.t.	11.5	n.t.	3.7	1.79	0.15
HPA-2a	>99.9	n.t.	n.t.	96.8	n.t.	n.t.	n.t.
HPA-2b	13.2	n.t.	n.t.	32.8	25.4	n.t.	n.t.
HPA-3a	80.9	n.t.	87.3	n.t.	78.9	72.9	78.6
HPA-3b	69.8	n.t.	n.t.	n.t.	70.7	80.7	71.9
HPA-4a	>99.9	n.t.	>99.9	n.t.	99.9	>99.4	>99.9
HPA-4b	0.0	n.t.	1.6	n.t.	1.7	0.6	0.17
HPA-5a	99.0	n.t.	n.t.	95.6	n.t.	>99.4	99.3
HPA-5b	19.7	n.t.	n.t.	37.6	n.t.	9.3	17.7
HPA-6bW	0.7	n.t.	n.t.	n.t	4.8	n.t.	n.t.

*Reference 5. †Zhang H. Dissertation for Post Grad Diploma in Applied Science 1999 (unpublished observations)

NAIT and HLA class II associations

The study of maternal HLA genotypes in women with a history of NAIT has shown that the genetic polymorphism of the β chains of class II molecules plays an important part in the capacity to form HPA antibodies (13). In a study by Williamson et al, anti-HPA-1a was detected in 46 of 387 (12%) pregnant HPA-1a negative women. All but one were HLA-DRB3*0101 (an allele of HLA-DR52) positive (30). Although the importance of binding between GPIIIa-derived peptides and HLA-DRB3*0101 molecules increased the risk of alloimmunisation by a factor of 140, its positive predictive value was only 35%. Therefore, 65% of those who were HPA-1a negative and DRB3*0101 positive did not become immunised. Some of their babies (15%) would themselves be HPA-1a negative. The overall frequency of the DRB3*0101 allele in the study was 31.9%, similar to the frequency in Caucasians. However, the negative predictive value of 99.6% has helped to clarify its potential usefulness in a screening programme, because mothers who are HPA-1a negative and DRB3*0101 negative have a very low risk for alloimmunisation. HLA-DQB1*0201 (an allele of HLA-DQ2), has also been reported to have an increased incidence in cases of NAIT due to anti-HPA-1a (45). A case report that demonstrates these associations is presented in the appendix.

Conversely, mothers with AITP and an HLA-DRB3* genotype (linked with HLA-DR11, 12, 13, 14, 17 and 18) seem to be protected against giving birth to a thrombocytopenic newborn (13). There is no known association between response to the HPA-1b form of platelet glycoprotein and the HLA-DRB3* genotype.

Anti-HPA-5b alloantibodies are common in pregnant women and formed mostly by HLA-DR6 positive individuals, which includes the DRB1*13 and DRB1*14 alleles, indicating that HLA class II determinants are predictive for the development of maternal antibodies, but not for any deleterious effects of these antibodies in the neonate (32), an important distinction. Approximately 69% of Caucasoid mothers with anti-HPA-5b were found to be positive for HLA-DR6 (37).

Other factors associated with disease severity

Previously, it has been thought that there was no clear correlation between the titre of anti-HPA-1a antibodies and severity of fetal thrombocytopenia (1,46). A recent study, however, reported a significant association between severe thrombocytopenia and a third trimester antibody titre of 32 or greater using the MAIPA assay (positive predictive value of 75%) (30).

There have been reports that maternal platelet antibody levels often decrease during gestation, even in severe cases of NAIT (46-48). Haemodilution may contribute to this effect, but it does not appear to be a major factor (48). Neither is the IgG subclass produced an indication of severity of thrombocytopenia (46,49). Most sera in those studies contained IgG1 alone or in combination with IgG3, but no significant difference between those giving birth to babies with or without NAIT.

Other factors which could affect the disease severity may include the glycosylation of maternal antibodies, the structure, site density, maturational development and tissue distribution (including megakaryocytes) of fetal platelet and platelet-specific antigens, the efficiency of IgG transport to the fetus, the functional maturity of the fetal reticuloendothelial system, polymorphisms which affect Fcγ receptor function, and the presence of anti-HLA related inhibitory antibodies which block the receptor FcγR function on fetal macrophages and inhibit phagocytosis (50).

The mechanism of reciprocal feto-maternal immunomodulation is being studied with the aid of a placental lobule perfusion model, to see whether some fetuses are able to control maternal antibody production via placental transfer of downregulatory agents such as major histocompatibility complexes, immunoglobulin factors or cytokines (47).

Laboratory testing

Diagnosis of NAIT is straightforward if an HPA-specific alloantibody is detected in the maternal serum and the corresponding antigen on the fetal/paternal platelets. However, variable results may be obtained depending on the specificity of the antibody and the sensitivity of the method used. It is important to differentiate reactions due to HLA antibodies, and to exclude a maternal platelet autoantibody or transfusion-induced alloantibody. Participation in external quality assurance programmes, such as the annual Australasian Platelet Antibody Workshop coordinated by the Australian Red Cross Blood Service, facilitates a more standardised approach to the investigation of platelet antigens and antibodies.

Recent evidence suggests that a fetus who survives NAIT often has negligible levels of the alloantibody within its circulation (47), therefore testing of the maternal serum is important. Platelets from the father should be included with the panel of group O donors for identification of the maternal platelet alloantibody in cases when a new, rare or private antigen is implicated (7).

Techniques for detecting and identifying platelet antibodies and alloantigens

A brief description of investigative techniques employed in platelet immunology relating to NAIT is presented. The presence of complement dependent maternal HLA antibodies can be detected by the standard microlymphocytotoxicity test (LCT) (51). Typing sera have to be free of HLA antibodies due to the strong expression of HLA antigens on platelets.

Serologic typing of platelets

Methods using whole (intact) platelets include:

1. Platelet immunofluorescence test (PIFT) either as a suspension or adhesion test. Reading may be performed microscopically or by flow cytometry (23,47), and chloroquine (52) will remove the HLA antigens.

2. Different enzyme-linked immunosorbent assay (ELISA) techniques have been established and are partly commercially available (23,53).

3. The mixed passive haemagglutination technique (MPHA) may produce very good results when used in experienced laboratories (54). MPHA was about 20 times more sensitive than the LCT in detecting IgG HLA antibodies. Pretreatment of platelets with chloroquine is used to elute β2-microglobulin and thereby abolish the reactivity of HLA antibodies in MPHA and immunofluorescence tests (52).

4. The solid-phase red-cell adherence (SPRCA) method as described by Lown (55) provides a simple and rapid platelet antibody screening technique with similar sensitivity to the PIFT. The platelets can be treated with chloroquine (52) to remove the HLA antigens.

Glycoprotein-specific methods

These circumvent the problem of contaminating HLA antibodies in typing reagents.

5. The monoclonal antibody-specific immobilisation of platelet antigen (MAIPA) assay is a quantitative capture assay using microwell plates and an ELISA indicator system (56), and has a high sensitivity which allows reliable typing of antigens that are expressed only in low

numbers on the platelet membrane, e.g., HPA-5 (23). However, one of the major disadvantages is the existence of false negative results due to competition between the human antibody and monoclonal antibodies (Mo-Abs) for the same or closely related epitopes (57). The MAIPA assay is more sensitive than the standard LCT in detecting platelet-reactive HLA antibodies (58).

6. The modified antigen-capture enzyme-linked immunosorbent assay (MACE) (59). A sensitive binding assay, such as the platelet immunofluorescence test, should be combined with an antigen capture method, e.g., (MAIPA), to detect weak antibodies or antibodies that react with relatively few antigen sites, such as anti-HPA-5b. This is especially important if multispecific HLA antibodies are also present (14).

7. Immunochemical techniques can be used to characterise new alloantigens. For example, immunoprecipitation was used to detect the antigens of the Gov system (23). Immunoblotting can be used to identify the underlying protein by size and immuno-specificity (23,57).

Genomic typing

Since the molecular genetics of 16 platelet polymorphisms have been elucidated,

genotyping is possible.

8. Restriction fragment length polymorphism (RFLP) analysis was the first DNA based typing assay used because in the majority of cases the single base substitution is associated with a cleavage site for a restriction endonuclease. Genomic DNA is amplified by the polymerase chain reaction (PCR) with primers encompassing the polymorphic region. The amplified DNA is then exposed to an allele-specific endonuclease and the fragments are separated on agarose gel (23).

9. Sequence-specific oligonucleotide hybridisation (SSO) has been used for the confirmation of HPA-4 polymorphisms due to the lack of suitable restriction sites. PCR amplified DNA encompassing the polymorphic region is fixed to a nylon membrane and then hybridised with labelled allele-specific oligonucleotide probes and an appropriate detection system (23).

10. Sequence-specific primers (SSP) have been applied mainly to the diallelic antigen systems. Primer design entails matching of the 3' end with the allele to be detected, and a mismatch inhibits DNA amplification under the appropriate conditions. The PCR products are analysed on agarose gel and the pattern of positive and negative reactions determines the HPA type (23).

Comparison of platelet antigen phenotyping and genotyping has to take into account that biological variations of the encoding genes may also lead to discrepant test results (23).

Treatment of NAIT

The nadir of the platelet count in the affected neonate occurs 2-5 days after delivery, when the splenic circulation becomes established (3,4,14). If bleeding due to severe thrombocytopenia can be avoided during the first few days of life, affected infants recover without event over 1 to 2 weeks depending on the rate of disappearance of the maternal anti-platelet antibody from the neonatal circulation (2,60).

If platelet transfusions are required for the neonate, they should be compatible with the maternal alloantibody. The mother's platelets would meet this criteria, providing apheresis platelet collection is available and risk factors for infection have been excluded. Platelets from the mother collected in this way must be washed to remove the maternal plasma containing the antibody, resuspended in blood group AB plasma and irradiated to eliminate the risk of graft-versus-host disease (7,61). The occasional poor response is probably caused by alterations of platelet function during the washing procedure (3). Alternatively, transfusion centres should be able to provide ABO compatible and HPA-1a antigen- negative donor platelets, suitable for most common cases of NAIT.

Win reported two cases of NAIT in which two infants with pre-transfusion platelet counts of 17 and 9 (10^9/L responded to random-donor platelet transfusion (61). Subsequent investigation revealed that both mothers were HPA-1a negative and anti-HPA-1a alloantibodies were detected in their serum. Both fathers were homozygous for HPA-1a antigen and both donors were typed as HPA-1a positive. The report postulates that the transfused antigen-positive platelet load might have overwhelmed the anti-HPA-1a antibody in the infants' circulations, resulting in survival of subsequently transfused platelets and allowing the recovery of megakaryocytes by neutralising the anti-HPA-1a antibody. Therefore, random-donor platelet transfusion could be given in urgent situations while waiting for matched platelets.

Mercier et al reported the results of platelet transfusions in a neonate with NAIT caused by anti-HPA-1b. The patient was transfused with a random platelet pool (3/4 of donors are compatible) and this was found to be as effective as selected single-donor platelet units (33).

Intravenous immunoglobulin (IVIG) administration to the neonate can be considered only when haemorrhage is not obvious, because the effect is delayed for 12-18 hours after injection. In clinically silent ICH, monitoring the platelet count, and performing cranial and abdominal ultra-sound is recommended for evidence of neurological impairment (7). In subsequent pregnancies, prenatal diagnosis by fetal blood sampling at 20-22 weeks gestation is recommended by some (23), and prevention of ICH during pregnancy and delivery can be attempted by either weekly maternal injection of high doses of IVIG with or without corticosteroids, or weekly intrauterine platelet transfusions with antigen-negative platelets (7). The conservative treatment of a single case of NAIT at the recommended dosage of 1g/kg of patient's weight/week of IVIG from 28-38 weeks of pregnancy will consume at least 600g of IVIG for a patient weighing 60 kg (47). The preparation of IVIG products such as Intragam requires a large pool of human plasma donated by voluntary blood donors, so there is the potential for demand to outstrip supply, resulting in rationing and priority measures.

Also, the optimal antenatal therapy to reverse fetal alloimmune thrombocytopenia has not yet been determined (7). In a study of 37 high-risk pregnancies involving anti-HPA-1a immunisation, the overall outcome for mothers treated with IVIG and those with steroids was unpredictable with a therapy failure rate of 33% (11). In pregnancies at risk, mothers should be advised to avoid drugs that interfere with platelet function (e.g., aspirin, antibiotics) (7).

Cost effectiveness of a screening programme

NAIT is potentially preventable. Currently there is no routine antenatal screening, with the first affected infant not usually diagnosed before birth. In these cases there will be a risk of severe NAIT. Subsequent pregnancies can be monitored and treated accordingly. When the incidence of severe fetal/neonatal morbidity or mortality due to NAIT of 10-20/100,000 live births (9) is compared to a mortality of 6/100,000 live births from Rh D haemolytic disease (62), a screening programme equal to that for HDN should be considered. A study published in 1998 is referred to by Bessos et al, which indicated that it cost £125,000 to bring up a child with severe disability from birth to 17 years (9). An economic analysis is only as good as the clinical information and data available, and in FMAIT there is still uncertainty about the incidence of morbidity and mortality and the effectiveness of the interventions

available. However, as Murphy et al point out, a screening programme does not have to save money to be worthwhile (63). A screening programme should fulfil the criteria set out by the World Health Organisation (WHO). These screening principles are listed with a brief discussion in relation to NAIT (63,64).

1. The condition should be an important health problem.

This can be defined either by population prevalence, or by a severe outcome in untreated cases. Severe thrombocytopenia has been reported in 1 in 1200 neonates, and if ICH has occurred, then blindness and major physical and intellectual disability can result.

2. The natural history should be well understood.

Maternal alloimmunisation to HPA-1a is the commonest cause of NAIT in Caucasian women, particularly in association with HLA-DRB3*0101. Unfortunately, there is no reliable predictor of severe clinical disease, such as antibody titres or subclass.

3. There should be a recognisable early stage.

This could be defined as antibodies without fetal thrombocytopenia, mild thrombocytopenia, or severe thrombocytopenia without neurological sequelae.

4. Treatment at an early stage should be more effective than treatment at a later stage.

Antenatal treatment options include IVIG to the mother, but one third of such infants still have a birth platelet count of $<50 \times 10^9$/L; or intrauterine transfusions of selected platelets to the fetus with a 0.5-1% risk of cord haemorrhage. Postnatally, the choice is between IVIG and selected platelets, depending on the platelet count. There are no primary prevention interventions available for FMAIT.

5. There should be a suitable test.

Antenatal screening by a combination of HPA-1 typing, HPA-1a antibody detection and HLA class II typing would identify pregnancies at high risk of NAIT caused by anti-HPA-1a.

6. The test should be acceptable to the population.

In the study of Williamson et al, 74% of HPA-1a negative women agreed to enrol for further investigation. All women chose to be managed conservatively, although knowledge that antibodies were present increased the Caesarean section rate to 36% (30).

7. There should be adequate facilities for the diagnosis and treatment of abnormalities detected.

Screening might generate a number of women requiring fetal blood sampling. The timing of this is a balance between being early enough to prevent ICH, and late enough to allow delivery of a mature infant if there is unexpected haemorrhage from the cord.

8. The chance of physical or psychological harm to those screened should be less than the chance of benefit.

This has not yet been established for NAIT. It has been estimated that, taking ICH as an endpoint, a randomised study comparing conservative management with invasive therapy would require one million pregnancies to be screened initially to obtain a definitive answer as to whether prevention of ICH by screening is not only possible, but cost-effective.

Discussion

The question is whether routine screening for FMAIT caused by anti-HPA-1a alloantibodies would be advantageous. Typing pregnant women for HPA-1a would be a logical first screening step, since approximately 97% are HPA-1a positive and can be excluded from further investigation. HLA typing for the DRB3*0101 allele would identify those at the greatest risk of alloimmunisation. Testing for anti-HPA-1a antibodies at 26, 30 and 36 weeks of those who are HLA-DRB3*0101 positive, and at 28 and 34 weeks for those who were negative has been suggested (30). As the costs of HLA typing are considerable, screening all HPA-1a negative women for HPA-1a antibodies would be less costly, although further improvements in assay performance are required.

The main aim of antenatal screening for FMAIT would be to identify potentially severe cases, and to provide treatment to prevent antenatal ICH. It is recognised that subsequent pregnancies of HPA-1a alloimmunised women with a history of a previously affected infants are associated with a high risk of recurrence and a poor outcome. Those with no past history of NAIT but in whom anti-HPA-1a antibodies have been found are less likely to have had a severe ICH in *utero*.

Unfortunately, there is no reliable indicator of severe clinical disease which might be used to select pregnancies for aggressive intervention. One of the difficulties of antenatal screening is that the only way of determining the fetal platelet count is to perform fetal blood sampling to measure the platelet count, a procedure which has the potential to cause fetal loss from cord haemorrhage and to boost maternal antibody titres.

There are no controlled trials showing that antenatal screening for FMAIT reduces morbidity or mortality. It is recognised that ICH due to NAIT can produce devastating clinical effects, but further research is required to identify factors useful for predicting severe disease, and the most appropriate approach to antenatal therapy in women with anti-HPA-1a but no previous history of affected pregnancies. Further work is also needed to determine the optimal antenatal management of subsequent pregnancies.

Conclusions

NAIT is an under-recognised and under-diagnosed syndrome with a risk of mortality or permanent neurological damage as a result of ICH. Caucasians have an increased incidence of NAIT due to anti-HPA-1a alloantibody, but more research is required before routine antenatal screening can be recommended. Postnatal screening can be achieved by carrying out a platelet count on a cord blood sample, but this is unlikely to prevent the serious consequences from ICH in severe cases of NAIT. However, there is the potential to improve the postnatal treatment of NAIT, and any delays or difficulties in the laboratory confirmation of the diagnosis should not prevent treatment of an infant with bleeding or severe thrombocytopenia. Rapid availability of HPA-1a and HPA-5b-negative platelet concentrates will be compatible in most cases. Often, only one transfusion of compatible platelets is sufficient, as the thrombocytopenia is self-limiting (63). But one infant was reported to have had multiple platelet transfusions and IVIG over the 82 days it took for his platelet count to recover (30). This was thought to relate to the presence of ICH.

Maori and Polynesian mothers do not appear to have the same risk factors.

Future directions

There is an urgent need for a non-invasive, simple investigation to indicate the severity of fetal thrombocytopenia in an affected infant with FMAIT (47). It may be possible in the future to carry out fetal platelet typing using samples of maternal plasma, as has been shown for fetal Rh(D) typing. Unlike anti-D, an anti-HPA-1a immunoglobulin preparation is not commercially available at present for a passive immunoprophylaxis programme (6).

Monoclonal antibodies against platelet alloantigens obtained from phage display libraries will circumvent the shortage of human derived typing sera, and supply both diagnostic and therapeutic reagents (65).

Research is being done on the identification and analysis of the clinically effective component/s of maternal as well as infused pooled gamma globulin products because a better understanding of the mode

of action of IVIG will hopefully allow the production of specifically effective antibodies with monoclonal engineering techniques, so preventing the transfusion of unnecessary IgG protein molecules into susceptible patients (47).

One forecast for the future is certain - there will be continued change in technology and knowledge in the field of platelet immunology, which will lead to improvements in diagnostic testing for NAIT, and also for patient care.

Appendix

Case Report

The baby of Mrs X was delivered by caesarian section at 35 weeks gestation. The baby had spontaneous bleeding and a platelet count of less than 10 (10^9/L. A transfusion of random platelets was given on the second day which raised the baby's platelet count to 307 (10^9/L. This was Mrs X's third pregnancy, and unfortunately an ultrasound revealed an intracranial haemorrhage and the baby subsequently died. Relevant laboratory results of the maternal post natal specimens were as follows:

Maternal platelet count:	normal
HLA class I antibody:	anti-HLA-A9+
Platelet-specific alloantibody:	anti-HPA-1a
Platelet antigen phenotype:	HPA-1a negative
Platelet antibody titre:	64 by SPRCA
HLA-DR Typing by DNA PCR-SSO:	HLA-DRB1*03011/0305, *14; DRB3*0101, *02

Mrs X generously donated her serum for screening blood donors as potential platelet donors for use in cases of neonatal alloimmune thrombocytopenia due to anti-HPA-1a antibodies, and also for typing of mothers with thrombocytopenic infants to determine their HPA-1a antigen status.

References

1. Kaplan C, Daffos F, Forestier F, Cox WL, Lyon-Caen D, Dupuy-Montbrun MC, et al. Management of alloimmune thrombocytopenia: antenatal diagnosis and in utero transfusion of maternal platelets. *Blood* 1988; 72: 340-3.

2. McFarland JG. Fundamentals and applications of molecular technology platelet alloantigen genotyping in clinical practice. *The Compendium of the American Association of Blood Banks 52nd Annual Meeting* 1999; 336-42.

3. Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, et al. 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989; i: 363-6.

4. Dreyfus M, Kaplan C, Verdy E, Schlegel N, Durand-Zaleski I, Tchernia G. Frequency of immune thrombocytopenia in newborns: a prospective study. Immune Thrombocytopenia Working Group. *Blood* 1997; 89: 4402-6.

5. von dem Borne AE, de Haas M, Simcek S, Porcelijn L, van der Schoot CE. Platelet and neutrophil alloantigens in clinical medicine. *Vox Sang* 1996; 70 (Suppl. 3): 34-40.

6. Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Décary F. Alloimmunization to the PI^{A1} platelet antigen: results of a prospective study. *Br J Haematol* 1990; 74: 209-15.

7. Kaplan C. Immune thrombocytopenia in the foetus and the newborn: diagnosis and therapy. *Transfus Clin Biol* 2001; 8: 311-4.

8. Freedman J. Immune destruction of circulating cells: immune destruction of platelets. *The Compendium of the American Association of Blood Banks 52nd Annual Meeting* 1999; 430-4.

9. Bessos H, Hofner M, Salamat A, Wilson D, Urbaniak S, Turner ML. An international trial demonstrates suitability of a newly developed whole-blood ELISA kit for multicentre platelet HPA-1 phenotyping. *Vox Sang* 1999; 77: 103-6.

10. Hohlfeld P, Forestier F, Kaplan C, Tissot JD, Daffos F. Fetal thrombocytopenia: a retrospective survey of 5,194 fetal blood samplings. *Blood* 1994; 84: 1851-6.

11. Kaplan C, Murphy MF, Kroll H, Waters AH. Feto-maternal alloimmune thrombocytopenia: antenatal therapy with IvIgG and steroids - more questions than answers. European Working Group on FMAIT. *Br J Haematol* 1998; 100: 62-5.

12. Gruel Y, Boizard B, Daffos F, Forestier F, Caen J, Wautier JL. Determination of platelet antigens and glycoproteins in the human fetus. *Blood* 1986; 68: 488-92.

13. Gandemer V, Kaplan C, Quelvennec E, Poulain P, Laurent MC, Semana G, et al. Pregnancy-associated autoimmune neonatal thrombocytopenia: role of maternal HLA genotype. *Br J Haematol* 1999; 104: 878-85.

14. Letsky EA, Greaves M. Guidelines on the investigation and management of thrombocytopenia in pregnancy and neonatal alloimmune thrombocytopenia. Maternal and Neonatal Haemostasis Working Party of the Haemostasis and Thrombosis Task Force of the British Society for Haematology. *Br J Haematol* 1996; 95: 21-6.

15. Valat AS, Caulier MT, Devos P, Rugeri L, Wibaut B, Vaast P, et al. Relationships between severe neonatal thrombocytopenia and maternal characteristics in pregnancies associated with autoimmune thrombocytopenia. *Br J Haematol* 1998; 103: 397-401.

16. Udom-Rice I, Bussel JB. Fetal and neonatal thrombocytopenia. *Blood Rev* 1995; 9: 57-64.

17. Herman J. Neonatal thrombocytopenia short topic. *The Compendium of the American Association of Blood Banks 52nd Annual Meeting* 1999; 137-41.

18. Garratty G. Immune destruction of circulating cells: mechanisms of immune cell destruction. *The Compendium of the American Association of Blood Banks 52nd Annual Meeting* 1999; 419-23.

19. Baxter-Lowe LA. Fundamentals and applications of molecular technology program: molecular HLA typing methods. *The Compendium of the American Association of Blood Banks 52nd Annual Meeting* 1999; 343-7.

20. van Loghem JJ Jr, Dorfmeijer H, van Hart M, Schreuder F. Serological and genetical studies on a platelet antigen (Zw). *Vox Sang* 1959; 4: 161-9.

21. Santoso S, Kiefel V. Human Platelet-specific alloantigens: update. *Vox Sang* 1998; 74 (Suppl. 2): 249-53.

22. Metcalfe P, Watkins NA, Ouwehand WH, Kaplan C, Newman P, Kekomaki R, et al. Nomenclature of human platelet antigens. *Vox Sang* 2003; 85: 240-5.

23. Kroll H, Kiefel V, Santoso S. Clinical aspects and typing of platelet alloantigens. *Vox Sang* 1998; 74 (Suppl. 2): 345-54.

24. Leeksma OC, Giltay JC, Zandbergen-Spaargaren J, Modderman PW, van Mourik JA, von dem Borne AE. The platelet alloantigen Zwa or PlA1 is expressed by cultured endothelial cells. *Br J Haematol* 1987; 66: 369-73.

25. Giltay JC, Brinkman H-JM, von dem Borne AE, van Mourik JA. Expression of the alloantigen Zwa (or PlA1) on human vascular smooth muscle cells and foreskin fibroblasts: a study on normal individuals and a patient with Glanzmann's thrombasthenia. *Blood* 1989; 74: 965-70.

26. Warwick RM, Vaughan J, Murray N, Lubenko A, Roberts I. In vitro culture of colony forming unit-megakaryocyte (CFU-MK) in fetal alloimmune thrombocytopenia. *Br J Haematol* 1994; 88: 874-7

27. Giltay JC, Brinkman H-JM, Vlekke A, Kiefel V, van Mourik JA, von dem Borne AE. The platelet glycoprotein Ia-IIa-associated Br-alloantigen system is expressed by cultured endothelial cells. *Br J Haematol* 1990; 75: 557-60.

28. Daffos F, Forestier F, Muller JY, Reznikoff-Etievant M, Habibi B, Capella-Pavlovsky M, et al. Prenatal treatment of alloimmune thrombocytopenia (Letter). *Lancet* 1984; 2: 632.

29. Goldberger A, Kolodziej M, Poncz M, Bennett JS, Newman PJ. Effect of single amino acid substitutions on the formation of the PI^{A} and Bak alloantigenic epitopes. *Blood* 1991; 78: 681-7.

30. Williamson LM, Hackett G, Rennie J, Palmer CR, Maciver C, Hadfield R, et al. The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PI^{A1}, Zw^{a}) as determined by antenatal screening. *Blood* 1998; 92: 2280-7.

31. Doughty HA, Murphy MF, Metcalfe P, Waters AH. Antenatal screening for fetal alloimmune thrombocytopenia: the results of a pilot study. *Br J Haematol* 1995; 90: 321-5.

32. Panzer S, Auerbach L, Cechova E, Fischer G, Holensteiner A, Kittl EM, et al. Maternal alloimmunization against fetal platelet antigens: a prospective study. *Br J Haematol* 1995; 90: 655-60.

33. Mercier P, Chicheportiche C, Reviron D, Dabanian C, Bourgue F, Chiaroni J, et al. Neonatal thrombocytopenia in HLA-DR, -DQ, -DP-typed mother due to rare anti-HPA-1b (PLA2) (Zwb) fetomaternal immunization. *Vox Sang* 1994; 67: 46-51.

34. von dem Borne AE, von Riesz E, Verheugt FWA, ten Cate JW, Koppe JG, Engelfriet CP, et al. Bak^{a}, a new platelet-specific antigen involved in neonatal allo-immune thrombocytopenia. *Vox Sang* 1980; 39: 113-20.

35. Boehlen F, Kaplan C, de Moerloose P. Severe neonatal alloimmune thrombocytopenia due to Anti-HPA-3a. *Vox Sang* 1998; 74: 201-4.

36. McGrath K, Minchinton R, Cunningham I, Ayberk H. Platelet anti-Bak^{b} antibody associated with neonatal alloimmune thrombocytopenia. *Vox Sang* 1989; 57: 182-4.

37. Kaplan C, Morel-Kopp MC, Kroll H, Kiefel V, Schlegel N, Chesnel N, et al. HPA-5b (Br^{a}) neonatal alloimmune thrombocytopenia: clinical and immunological analysis of 39 cases. *Br J Haematol* 1991; 78: 425-9.

38. Corral J, Rivera J, González-Conejero R, Vicente V. The number of platelet glycoprotein Ia molecules is associated with the genetically linked 807 C/T and HPA-5 polymorphisms. *Transfusion* 1999; 39: 372-8.

39. Kiefel V, Santoso S, Katzmann B, Mueller-Eckhardt C. The Br^{a}/Br^{b} alloantigen system on human platelets. *Blood* 1989; 73: 2219-23.

40. Engelfriet CP, Reesink HW, Bussel J, Godeau B, Bierling P, Panzer S, et al. The treatment of patients with autoimmune thrombocytopenia with intravenous IgG-anti-D. *Vox Sang* 1999; 76: 250-5.

41. Kaplan C, Morel-Kopp MC, Clemenceau S, Daffos F, Forestier F, Tchernia G. Fetal and neonatal alloimmune thrombocytopenia: current trends in diagnosis and therapy. *Transfus Med* 1992; 2: 265-71.

42. Ohto H, Morita S, Tohyama Y, Noguchi M, Motoki R, Sato A. Anti-platelet antibodies in pregnant women and neonatal thrombocytopenia: a prospective study. Neonatal Thrombocytopenia Study Group. *Transfusion* 1994; 34 (Suppl. 10): S319.

43. Ikeda H, Mitani T, Ohnuma M, Haga H, Ohtzuka S, Kato T, et al. A new platelet-specific antigen, Nak^{a}, involved in the refractoriness of HLA-matched platelet transfusion. *Vox Sang* 1989; 57: 213-7.

44. Kankirawatana S, Kupatawintu P, Juji T, Veerakul G, Ngerncham S, Chongkolwatana V, et al. Neonatal alloimmune thrombocytopenia due to anti-Nak^{a}. *Transfusion* 2001; 41: 375-7.

45. Anolik JH, Blumberg N, Snider J, Francis CW. Posttransfusion purpura secondary to an alloantibody reactive with HPA-5a (Br^{b}). *Transfusion* 2001; 41: 633-6.

46. Proulx C, Fillion M, Goldman M, Bradley A, Devine D, Décary F, et al. Analysis of immunoglobulin class, IgG subclass and titre of HPA-1a antibodies in alloimmunized mothers giving birth to babies with or without neonatal alloimmune thrombocytopenia. *Br J Haematol* 1994; 87: 813-7.

47. Dawkins B, Minchinton R, Maguire D, Addison R. Human feto-maternal immunomodulation during pregnancy. *Topics in Transfusion Medicine, Australasian Society of Blood Transfusion Inc* 1999; 6: 14-6.

48. Dawkins B. Monitoring anti-HPA-1a platelet antibody levels during pregnancy using the MAIPA test. *Vox Sang* 1995; 68: 27-34.

49. Kurz M, Stöckelle E, Eichelberger B, Panzer S. IgG titer, subclass, and light-chain phenotype of pregnancy-induced HPA-5b antibodies that cause or do not cause neonatal alloimmune thrombocytopenia. *Transfusion* 1999; 39: 379-82

50. Hadley AG. A comparison of in vitro tests for predicting the severity of haemolytic disease of the fetus and newborn. *Vox Sang* 1998; 74 (Suppl. 2): 375-83.

51. Mittal KK. Standardization of the HLA typing method and reagents. *Vox Sang* 1978; 25: 275-9.

52. Nordhagen R, Flaathen ST. Chloroquine removal of HLA antigens from platelets for the platelet immunofluorescence test. *Vox Sang* 1985; 48: 156-9.

53. Sintnicolaas K, van der Steuijt KJ, van Putten WL, Bolhuis RL. A microplate ELISA for the detection of platelet alloantibodies: comparison with the platelet immunofluorescence test. *Br J Haematol* 1987; 66: 363-7.

54. Shibata Y, Juji T, Nishizawa Y, Sakamoto H, Ozawa N. Detection of platelet antibodies by a newly developed mixed agglutination with platelets. *Vox Sang* 1981; 41: 25-31.

55. Lown JA, Ivey JG. Evaluation of a solid phase red cell adherence technique for platelet antibody screening. *Transfus Med* 1991; 1: 163-7.

56. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C. Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. *Blood* 1987; 70: 1722-6.

57. Kaplan C. Evaluation of serological platelet antibody assays. *Vox Sang* 1998; 74 (Suppl. 2): 355-8

58. Kurz M, Knöbl P, Kalhs P, Greinix HT, Höcker P, Panzer S. Platelet-reactive HLA antibodies associated with low posttransfusion platelet increments: a comparison between the monoclonal antibody-specific immobilization of platelet antigens assay and the lymphocytotoxicity test. *Transfusion* 2001; 41: 771-4.

59. Menitove JE, Pereira J, Hoffman R, Anderson T, Fried W, Aster RH. Cyclic thrombocytopenia of apparent autoimmune etiology. *Blood* 1989; 73: 1561-9.

60. McFarland JG, Frenzke M, Aster RH. Testing of maternal sera in pregnancies at risk for neonatal alloimmune thrombocytopenia. *Transfusion* 1989; 29: 128-33.

61. Win N. Provision of random-donor platelets (HPA-1a positive) in neonatal alloimmune thrombocytopenia due to anti HPA-1a alloantibodies (Letter). *Vox Sang* 1996; 71: 130-1.

62. Whitfield CR, Raafat A, Urbaniak SJ. Underreporting of mortality from RhD haemolytic disease in Scotland and its implications: retrospective review. *BMJ* 1997; 315: 1504-5.

63. Murphy MF, Williamson LM, Urbaniak SJ. Antenatal screening for fetomaternal alloimmune thrombocytopenia: should we be doing it? *Vox Sang* 2002; 83 (Suppl. 1): 409-16.

64. Williamson LM. Screening programmes for foetomaternal alloimmune thrombocytopenia. *Vox Sang* 1998; 74 (Suppl. 2): 385-9.

65. Watkins NA, Ouwehand WH. Introduction to antibody engineering and phage display. *Vox Sang* 2000; 78: 72-9.

Psuedothrombocytosis caused by white blood cell fragments

Kevin A Taylor, BMLSc; Section Head, Core Haematology Laboratory, Canterbury Health Laboratories, Christchurch

Introduction

Spurious platelet counts generated from automated haematology analysers are a well-known occurrence within the routine laboratory. Artefactually low platelet counts are predominantly caused by fibrin strands/clots and platelet clumping (1). Less frequently the presence of neutrophil satellisim is an additional cause. The laboratory identification through routine blood smears on thrombocytopenic patients will avert needless clinical intervention on these patients with pseudothromboc ytopenias.

Less well known are instances where the platelet count is artefactually raised. Numerous causes for these pseudothrombocytosis have been documented including red cell fragments, cryoglobulinaemia (1), malarial parasites (2), yeast cells (3) and white cell fragments (4-6). Clinically the presence of a falsely raised platelet count may be of much greater significance as it may shift the platelet count outside the region in which platelets may normally be transfused, resulting in possible severe clinical consequences (6).

Case report

A 23 year old female presented to her general practitioner with fever and a sore throat. The initial complete blood count had a raised white blood cell count of 156.5 x 10^9/L, haemoglobin of 118 g/L and platelet count of 31 x 10^9/L. Examination of the blood film showed a large number of blast cells with myeloid linage morphology and a diagnosis of acute myeloid leukaemia was made.

Flow cytometry performed upon the peripheral blood showed 80% immature myeloid cells expressing weak CD45, the stem cell antigen CD34, weak expression HLA DR, CD9 and the myeloid antigens CD117, CD13, CD15 (partial only), CD33 and myeloperoxidase (proenzyme), with aberrant expression of CD2. The cells were negative for the expression of myelomonocytic antigens.

Further examination of the blood film showed the presence of small corpuscles of similar size to a platelet (Figure 1). These corpuscles showed staining characteristics that were similar to the cytoplasm of the circulating blast cells in the peripheral blood. Blood films stained from different samples in different laboratories both showed these abnormal corpuscles confirming they were not a staining artefact.

A subsequent sample (WBC 181 x 10^9/L, Hb 97 g/L, Plts 64 x 10^9/L) also showed the abnormal corpuscles. A myeloperoxidase stain was performed upon this sample. A number of the platelet sized corpuscles in the myeloperoxidase stain displayed blue granules suggesting myeloperoxidase positivity, rather than a red colour without granules that would normally be expected from platelets (Figure 2). A normal control showed no myeloperoxidase positivity in any of its platelet-sized corpuscles.

Four experienced morphologists carried out a manual count on upon the myeloperoxidase film to identify the percentage of positive corpuscles, which were of similar size to platelets. 15.5% (standard deviation 1.7) of the platelet sized corpuscles showed myeloperoxidase positivity. On the assumption that the myeloperoxidase positive corpuscles were not platelets, we calculated a corrected platelet count of 54 x 10^9/L.

Enumeration of the platelets using flow cytometry was performed on this subsequent sample using CD61 and CD41 specific antibodies and forward scatter (7,8). This method gave a platelet count of 53 X 10^9/L, which was lower than the results that were obtained from the automated analyser, but agrees with the count calculated from the myeloperoxidase stained blood film. The subsequent sample was also tested on the flow cytometer using an antibody specific for CD33. This showed a region in the same position as platelets on forward scatter that was distinct from both the white cell and red cell populations. However, the absolute value of CD33 positive corpuscles (3.2 x 10^9/L) did not make up the difference between the platelet count obtained

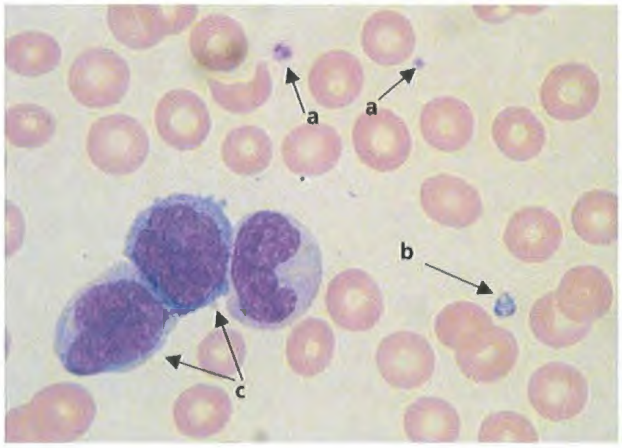


Figure 1. (a) Platelet. (b) Basophilic corpuscle of similar size to platelet with staining characteristics similar to the cytoplasm of the immature myeloid cells. (c) Immature myeloid cells

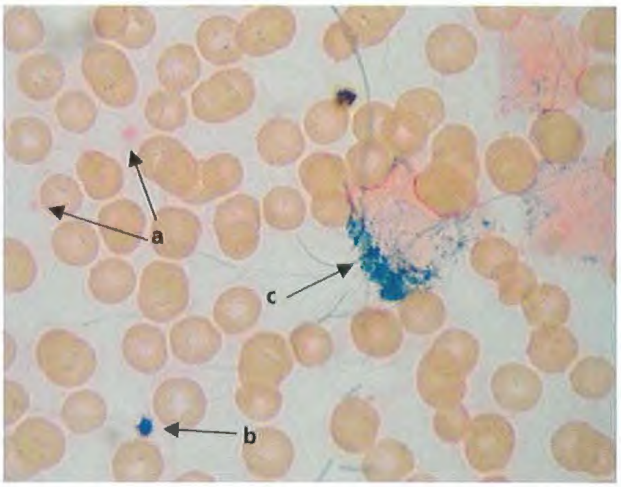


Figure 2. (a) Platelets, myeloperoxidase negative. (b) Platelet sized corpuscle, myeloperoxidase positive. (c) Myeliod cell, myeloperoxidase positive.

via flow and the platelet count from the automated analyser.

The abnormal platelet sized corpuscles were still present (in reduced numbers) after 5 days, by which stage the white cell count had dropped to 1.2 x 10^9/L (manual differential blast count 8%).

The results from the myeloperoxidase stain and the flow cytometry finding corpuscles that were CD33 positive suggest that the corpuscles observed in the peripheral blood were of white cell origin, presumably white cell fragments.

Discussion

White cell fragments have been reported predominantly in patients with haematological malignancies (4-6,9) which suggests that their most likely source is from the malignant cells circulating in the peripheral blood.

The most comprehensive study relating to the frequency of white cell fragments was done by van der Meer and colleagues (4). Their study showed 25% of patients (169 patients at initial diagnosis or relapse; 60 ALL, 81 AML [covering all FAB morphologies], 28 acute leukaemia type not specified) had pseudoplatelets in their blood smears. The corrected platelet counts meant that 4.1% of patients needed to be re-classified in terms of possible bleeding risk.

The appearance of white blood cell fragments had not previously been noted within our laboratory. This would suggest that other instances in which white cell fragments were present have been under diagnosed leading to falsely elevated platelet counts being reported through the haematology laboratory. This will be of clinical significance in leukaemic patients when assessing the need for platelet transfusions.

References

1. Bain BJ. Blood Cells: A Practical Guide (2nd Ed). Blackwell Science, London, 1995.

2. Crabbe G, van Poucke M, Cantinieaux B. Artefactually-normal automated platelet counts due to malaria-infected RBC. *Clin Lab Haemol* 2002; 24: 179-82.

3. Latif S, Veillon DM, Brown D, Kaltenbach J, Curry S, Linscott AJ, et al. Spurious automated platelet count. Enumeration of yeast forms as platelets by the cell-DYN 4000. *Am J Clin Pathol* 2003; 120: 882-85.

4. Van der Meer W, MacKenzie MA, Dinnissen JW, de Keijzer MH. Pseudoplatelets: a retrospective study of their incidence and interference with platelet counting. *J Clin Pathol* 2003; 56: 772-4.

5. Stass SA, Holloway ML, Peterson V, Creegan WJ, Gallivan M, Schumacher HR. Cytoplasmic fragments causing spurious platelet counts in the leukaemic phase of poorly differentiated lymphocytic lymphoma. *Am J Clin Pathol* 1979; 71: 125-8.

6. Hammerstrom J. Spurious platelet counts in acute leukaemia with DIC due to cell fragmentation. *Clin Lab Haematol* 1992; 14: 239-43.

7. Harrison P, Ault KA, Chapman S, Charie L, Davis B, Fujimoto K, et al. An interlaboratory study of a candidate reference method for platelet counting. *Am J Clin Pathol* 2001; 115: 448-59.

8. International Council for Standardization in Haematology Expert Panel on Cytometry, International Society of Laboratory Haematology Task Force on Platelet Counting. Platelet counting by the RBC/platelet ration method: a reference method. *Am J Clin Pathol* 2001; 115: 460-4.

9. Stass SA, Holloway ML, Slease RB, Schumacher HR. Spurious platelet counts in hairy cell leukaemia (Letter). *Am J Clin Pathol* 1977; 68: 530-1.

Address for correspondence: Kevin Taylor, Canterbury Health Laboratories, PO Box 151, Christchurch. Email: Kevin.taylor@cdhb.govt.nz.

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 for the April and August issue were Ross Hewett, LabPlus, Auckland; and Chris Sies, Canterbury Health Laboratories, Christchurch, for their articles "Drugs of abuse testing" *N Z J Med Lab Sci* 2004; 58: 9-12; and "Faecal occult blood testing: guaiac vs immunochemistry: which method should we use?" *N Z J Med Lab Sci* 2004; 58: 58-60.

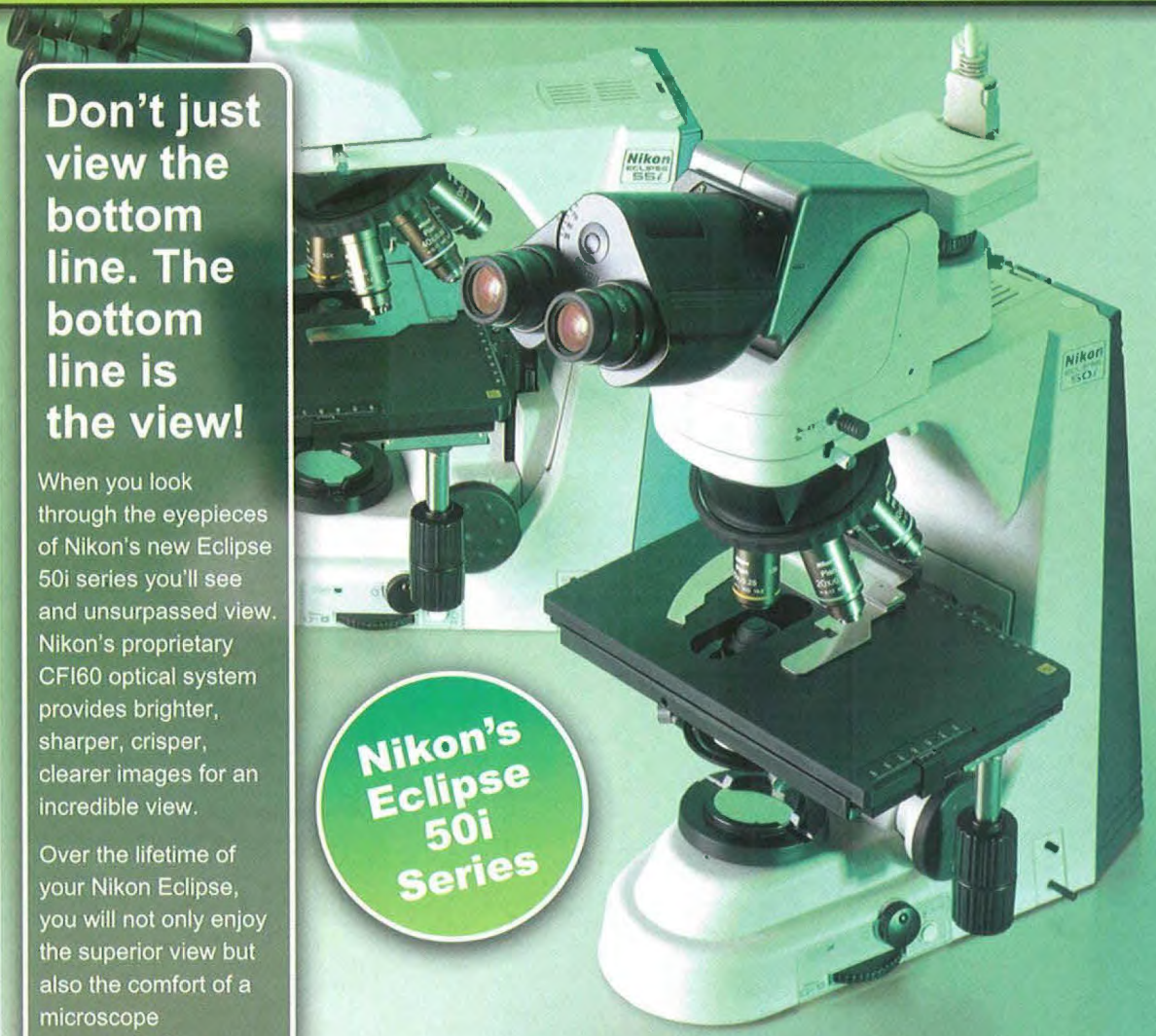
Planning to Purchase a Microscope?

Don't just view the bottom line. The bottom line is the view!

When you look through the eyepieces of Nikon's new Eclipse 50i series you'll see and unsurpassed view. Nikon's proprietary CFI60 optical system provides brighter, sharper, crisper, clearer images for an incredible view.

Over the lifetime of your Nikon Eclipse, you will not only enjoy the superior view but also the comfort of a microscope ergonomically designed to fit the user rather than force the user to fit the scope.

With a variety of accessories the Nikon Eclipse can be customised to match your ergonomic requirements, microscopy techniques and imaging needs.



Nikon's Eclipse 50i Series

< Some of the accessories for the 50i series >

Eye Level Risers

Put your phone books back to their proper use. The eye level risers allow you to raise the eye level up to 100mm. Allowing you to be in comfort while your scope is stable and secure on the bench.

Teaching heads

Allowing up to 5 multiple users to view the same preparation simultaneously.

Digital Cameras

Nikon have a range of digital cameras perfectly matched to their microscopes. Allowing you to configure your purchase to your specifications.

Auckland, New Zealand
T: (09) 267 2450
F: (09) 267 2451
Email: sales@coherent.com.au

Coherent SCIENTIFIC

Coherent Scientific Pty. Ltd., Inc. in South Australia, ACN 008 265 969, ABN 20 008 265 969

The diagnosis and treatment of prostate cancer: will commonsense prevail?

Brett Delahunt, ONZM, MD, FRCPA, Professor of Pathology and Molecular Medicine1;
David S Lamb, FRCR, FRANZCR, Head of Radiation Oncology and Clinical Senior Lecturer in Pathology and Molecular Medicine 1,2; John N Nacey MD, MBA, FRACS, Dean and Professor of Urology1,
Wellington School of Medicine and Health Sciences1 and2Wellington Hospital

Abstract

Adenocarcinoma of the prostate is currently the second most common malignancy and third most common cause of cancer-related deaths in males. The evidence to support systematic population based screening programmes is currently lacking, however, the role of the screening on demand is hotly debated.

Recent studies have shown that: PSA screening leads to a three fold increase in the detection of prostate cancer when compared to controls; survival decreases dramatically in patients after 15 years follow-up for initially untreated prostate cancer; radical prostatectomy leads to a 50% reduction in prostate cancer-specific deaths after 8 years follow-up when compared to those managed by "watchful waiting".

In this review, the cancer-screening controversy is discussed in the light of these recent discoveries and it is argued that screening for prostate cancer will be beneficial for selected men. In the first instance, patients who are at a high risk of developing prostate cancer due to a family history should be encouraged to undergo screening at regular intervals.

Key words: prostate adenocarcinoma, screening, prostate specific antigen, diagnosis, survival.

Introduction

As men age they become increasingly pre-occupied with their prostate gland. Despite this interest, few know where the gland is located and even fewer are aware of its function. What is well known, however, is that an enlarged prostate may produce "waterworks problems" and that in the older male prostate cancer is relatively common. Just how common prostate cancer is, is well illustrated in the recently published Ministry of Health Bulletin, *Cancer in New Zealand - Trends and Projections*, which shows it ranks as the second most common cancer and the third most common cause of cancer death in males (1). In 1997, 531 deaths were attributed to prostate cancer, and this number is projected to rise to 844 in 2012. To put these data into context, there were 643 deaths in females due to breast cancer in 1997, while projections for 2012 predict 774 deaths.

Despite the obvious morbidity and mortality related to prostate cancer, there is considerable debate regarding the necessity or even the desirability of screening to detect the disease in asymptomatic individuals.

Prostate cancer and its detection

Carcinoma of the prostate is an adenocarcinoma of the epithelium of prostatic lobules. Patients rarely present with urinary tract symptoms and the diagnosis is usually made following digital rectal examination (DRE) and measurement of serum prostate specific antigen (PSA). DRE is a simple procedure which allows assessment of the peripheral zone of the gland, where most clinically significant malignant tumours originate. While DRE is useful for the detection of malignancy in isolation, it has a relatively low sensitivity and moderate specificity as a diagnostic modality for prostate cancer. Any investigation for prostate cancer should include measurement of levels of serum PSA.

PSA is an organ-specific serine protease produced by the columnar epithelial cells that line the ducts and lobules of the prostate and periurethral glands (2,3). It is largely secreted into the lumen of the prostate ducts, and is found in high concentration in the seminal fluid, where it is involved in the liquefaction of the seminal coagulum (4). Normally a small proportion of PSA produced by the prostatic ductal system leaks into the prostate stroma and thence into the blood. Prostate cancer cells produce less PSA than normal prostate epithelial cells (2,5,6), but this is largely secreted into the stroma, and so presumably has much easier access to the blood stream.

PSA is a 28,400 Da glycoprotein (7) comprising 237 amino residues (8) with five inter-chain disulphide bonds and is approximately 8% carbohydrate in the form of a N-linked oligosaccharide side chain. In seminal plasma PSA exists in five isoforms, two of which are biologically active and differ in the degree of glycosylation, and three are biologically inactive "nicked" forms (9).

PSA can be measured in serum in two main forms: bound and free (10). PSA binds to a number of protease inhibitors, and most PSA in serum is complexed with alpha-1-antichymotrypsin (ACT). Minor fractions of PSA also exist bound to alpha-2-macroglobulin (A2M) and alpha-1-antitryspin (or protease) inhibitor (API). A small but variable amount of PSA in serum exists in free form (f-PSA). PSA has 5 major epitope domains, one of which is blocked by complexing to ACT. Antibodies to this domain are used to measure f-PSA. All commercial assays also measure the PSA-ACT and PSA-API complexes (11). However, none recognise the PSA-A2M complex, as the PSA molecule is interiorised by the altered conformation of the inhibitor (12). Total PSA (tPSA) in serum has a half-life of between 2.2 and 3.2 days (13,14) and clearance follows first order elimination kinetics.

Serum tPSA shows a gradual increase with age (15) and is also elevated in patients with prostate cancer and benign nodular hyperplasia. In many instances 4ng/ml is taken as the cut-off with patients having levels greater than this going forward for prostate biopsies in order to confirm or refute malignancy.

Patients with palpably abnormal glands or elevated tPSA usually undergo thin core biopsy of the prostate. This involves tissue sampling under ultrasound guidance, and typically between 6 and 15 cores of tissue, 1mm in diameter and 12 to 20mm in length are taken (16). The diagnostic yield of the core biopsy is greatly enhanced when more cores are taken and studies have shown the number of cancers detected to increase by 35% using a systematic five region approach, with the sampling of 13 cores when compared to the standard sextant (six core) biopsy (17). The absence of tumour tissue on a series of thin core biopsy specimens does not necessarily rule out a diagnosis of malignancy and in such circumstances clinical judgment plays an important role. It is usual for patients with negative biopsies to undergo a repeat procedure while tPSA levels are regularly monitored in order to determine if the PSA velocity is indicative of occult malignancy.

Although a screening process for the detection of prostate cancer, using a combination of DRE and tPSA measurement, would seem both rational and potentially effective, the critics of prostate cancer screening claim that there is no evidence that the process has any impact upon prostate cancer mortality. More specifically it has been claimed that screening, utilizing DRE and PSA measurement, is associated with significant numbers of false positive and false negative results and that the screening procedures and the treatment options for prostate cancer may themselves be associated with a significant morbidity/mortality that may outweigh any benefit to the patient. It is also claimed that many tumours are slow growing and may not cause symptoms or shorten life (18).

DRE/PSA screening

There is an extensive literature that investigates the validity of screening for the detection of prostate cancer with variable results. The consensus would, however, agree with the observations that the positive predictive value for tPSA >4.0ng/ml is only 42% and that this increases to 72% when DRE is also suggestive of malignancy. The positive predictive value is also substantially higher if tPSA >10 ng/ml is taken as the cut off (19).

The sensitivity of PSA as a marker of prostate cancer is complicated by the fact that benign prostatic tissue, as well as carcinoma, secretes the protein, however, while benign nodular hyperplasia of the prostate is usually associated with elevated levels of tPSA, these levels rarely exceed 10ng/ml. The validity of elevated tPSA as a cancer marker may be enhanced if an increasing level of the protein is observed over time (PSA velocity) and in this context an increase of the order of 2ng/ml/year is considered virtually diagnostic (20). It is also known that the fraction of free to total PSA (%fPSA) is increased to patients with prostate cancer and fractions <25% are considered abnormal. Percentage (%) fPSA has been shown to provide diagnostic data additional to PSA. Specifically it provides increased sensitivity for the diagnosis of malignancy in individuals with tPSA <4.0ng/ml and increased specificity in individuals with tPSA in the equivocal range of 4-10 ng/ml (21).

It must be realised that DRE/PSA screening is nothing more than that - a screening test that identifies individuals for whom additional study is appropriate. Critics argue that some cancers are PSA negative and that for these patients the screening process provides inappropriate reassurance. In reality PSA negative tumours are usually high grade and those are more likely to be detected by DRE, thus emphasizing the necessity of undertaking DRE and PSA testing in tandem. Not all breast cancers contain calcium on x-ray examination and yet mammography (which is itself associated with risks from excess radiation and has significant false positive and negative results) is strongly supported as a screening modality for breast cancer.

There is increasing evidence to show that systematic screening for prostate cancer does lead to the detection of occult cancers. A recent randomized Swedish study amongst 20,000 men screened over an eight year period showed a three-fold increase in the incidence of cancers detected in screened individuals compared to non-screened controls (22). It was also shown that tumours detected by screening were at a lower stage and grade than those diagnosed in unscreened patients. This later finding is of some significance to patient management as these patients would likely be suitable for radical prostatectomy, a procedure recently shown to halve the death rate amongst patients with prostate cancer (23). Treatment modality, as well as prognosis assessment, may also be assisted by determination of tPSA levels as a pre-treatment PSA velocity greater than 2ng/ml/year is associated with a significant risk of death, with tumour stage, Gleason score and tPSA level providing additional survival information (24).

How frequently is prostate cancer a clinically significant malignancy?

The 531 individuals who died of prostate cancer in New Zealand during 1997, would doubtless have had a strong opinion as to the malignant nature of the disease and yet, despite this obvious mortality, the report of the National Health Committee (18), which argues against screening for prostate cancer, contains the following;

> *"The majority of cases of prostate cancers are very slow-growing and not life-threatening, only a small minority of cases progress rapidly with invasion of surrounding tissues and distant metastases. Unfortunately, it is not possible to accurately determine which tumours are slow growing and which are aggressive."*

There are major errors in this statement and these relate to our understanding of the behaviour of prostate cancer and the ability of clinicians to predict this behaviour. In the past, the majority of prostate cancers that were diagnosed while confined to the prostate were discovered incidentally, following transurethral resection of prostate for relief of obstructive symptoms associated with benign nodular hyperplasia. In general these tumours did not metastasize and the belief grew that prostate cancer was an indolent disease that was unlikely to progress. In truth not all prostate cancers are created equal and it is now recognised that cancers in the peripheral zone of the prostate, which are diagnosed by needle biopsy, are very different in their behaviour than those of the central and transitional zones, which are sampled by transurethral resection (25). These peripheral zone cancers have a much more aggressive course and this is well illustrated by one study which showed 55% of all cases of low grade peripheral zone prostate cancer, diagnosed by needle biopsy to have extraprostatic extension at the time of radical prostatectomy (26).

In 1974 Donald Gleason proposed a grading and scoring system for prostate cancer, which was based on the architectural appearance of the tumour (27). Five grading categories were defined, with grade 1 being well differentiated and grade 5 being most poorly differentiated. As most prostate cancers consist of more than one grading pattern, Gleason proposed that each tumour should be assigned a numerical score, being the sum of the grades that constitute the greatest and second greatest volume of the tumour or by doubling the grade if only one architectural pattern was present. This means that each prostate cancer is now assigned to a Gleason score ranging from two to ten and to date numerous studies have validated the value of this scoring system in predicting tumour behaviour and patient outcome.

Recent studies have shown that over a long-term follow-up period prostate cancers do have the ability to advance and cause significant morbidity and mortality. The recently published study on the natural history of localized prostate cancer from Sweden showed that, regardless of initial stage and in both well differentiated and moderately differentiated tumours, the probability of progression from localised and indolent disease to metastatic disease, ultimately leading to tumour-related death, increased markedly in follow-up periods of greater than 15 years (28). Of particular concern, was the observation that the mortality was higher in those who were diagnosed at age 70 years or less.

These studies clearly demonstrate that prostate cancer is a serious form of malignancy that often requires intervention at the time of diagnosis. Data relating to long term recurrence further emphasise the desirability of initiating screening in younger males and in particular those with a family history, who are at higher risk of developing the cancer, and are more likely to develop the disease while relatively young (29).

Conclusions

Data adapted from the UK and presented in the National Health Committee document (18), suggests that of 1000 men less than 70 years of age undertaking PSA screening for prostate cancer, 136 will have elevated PSA and go on to prostate biopsy. Of these 103 will have negative biopsies, while 33 will be diagnosed with cancer. An additional 15 will have cancer which was not detected by the PSA test, while eight will have cancer that was missed on biopsy. What these data do not include, is the recognition that simultaneous DRE and assessment of %fPSA will increase the detection of prostate cancer in the group of individuals that are PSA negative on one original blood test. In addition, repeat biopsy will eventually lead to detection of the cancer in the eight individuals with occult malignancy who were negative on initial biopsy. What is not emphasised is that screening would lead to the detection of cancer in at least 33 and as many as 56 men who were in all probability asymptomatic at the time of diagnosis and who, within the next 15 to 20 years, would most likely have a painful death from metastatic prostate cancer. It is clear that many of the current arguments against prostate cancer screening are based upon old data and false premises and this emphasises the necessity of involving those who are active in the diagnosis and treatment of prostate cancer in the development of policies regarding the implementation of targeted screening programmes. The current blanket rejection of screening accords with the philosophy that if a tumour remains undetected then the difficult problem of deciding what is the appropriate treatment is avoided. This is reminiscent of the "If you can't win - don't try" philosophy of Homer Simpson.

The critics of population-based screening base their arguments on the apparent lack of evidence that screening produces any survival benefit and the assumption that most prostate cancers are not clinically significant. There is increasing evidence to show that early diagnosis and treatment of prostate cancer does impact upon patient survival, while it is clear that most cancers diagnosed on needle biopsy are clinically significant, especially those detected in patients under the age of 70 years. Patients with a family history of prostate cancer have up to an eleven fold risk of developing the disease and there can be little doubt that individuals in this group should be actively encouraged to undergo screening on a regular basis.

It is the role of the health practitioner to diagnose disease and treat accordingly, not to pretend that a disease is not present and hope it will remain clinically silent. Anti-screening policies relating to prostate cancer do nothing to reduce the community impact of what is, in reality, an important cause of mortality in males.

References

1. Cancer in New Zealand. Trends and Projections. Public Health Intelligence Occasional Bulletin No. 15. Ministry of Health, Wellington. pp338, 2002.

2. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. *Invest Urol* 1979; 17: 159-63.

3. Lilja H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *J Clin Invest* 1985; 76: 1899-903.

4. Schellhammer P, Wright GL Jr. Biomolecular and clinical characteristics of PSA and other candidate prostate tumor markers. *Urol Clin North Am* 1993; 20: 597-606.

5. Papsidero LD, Kuriyama M, Wang MC, Horoszewicz J, Leong SS, Valenzuela L, et al. Prostate antigen: a marker for human prostate epithelial cells. *J Natl Cancer Inst* 1981; 66: 37-42.

6. Qiu SD, Young CY, Bilhartz DL, Prescott JL, Farrow GM, He WW, et al. In situ hybridization of prostate-specific antigen mRNA in human prostate. *J Urol* 1990; 144: 1550-6.

7. Belanger A, van Halbeek H, Graves HC, Grandbois K, Stamey TA, Huang L, et al. Molecular mass and carbohydrate structure of prostate specific antigen: studies for establishment of an international PSA standard. *Prostate* 1995; 27: 187-97.

8. Lundwall A, Lilja H. Molecular cloning of human prostate specific antigen cDNA. *FEBS Lett* 1987; 214: 317-22.

9. Zhang WM, Leinonen J, Kalkkinen N, Dowell B, Stenman UH. Purification and characterization of different molecular forms of prostate-specific antigen in human seminal fluid. *Clin Chem* 1995; 41: 1567-73.

10. Ward AM, Catto JW, Hamdy FC. Prostate specific antigen: biology, biochemistry and available commercial assays. *Ann Clin Biochem* 2001; 38: 633-51.

11. Zhang WM, Leinonen J, Kalkkinen N, Stenman UH. Prostate-specific antigen forms a complex with and cleaves alpha 1-protease inhibitor in vitro. *Prostate* 1997; 33: 87-96.

12. Chen Z, Komatsu K, Prestigiacomo A, Stamey TA. Addition of purified prostate specific antigen to serum of female subjects: studies in the relative inhibition by alpha-2-macroglobulin and alpha-1-antichymotrypsin. *J Urol* 1996; 156: 1357-63.

13. Oesterling JE, Chan DW, Epstein JI, Kimball AW Jr, Bruzek DJ, Rock RC, et al. Prostate specific antigen in the preoperative and postoperative evaluation of localized prostatic cancer treated with radical prostatectomy. *J Urol* 1988; 139: 766-72.

14. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med* 1987; 317: 909-16.

15. Gray MA, Delahunt B, Fowles JR, Weinstein P, Cooke RR, Nacey JN. Assessment of ethnic variation in serum levels of total, complexed and free prostate specific antigen. Comparison of Maori, Pacific Island and New Zealand populations. *Pathology* 2003; 35: 480-3.

16. Delahunt B, Nacey JN. Thin core biopsy of prostate. *Pathology* 1998; 30: 247-56.

17. Eskew LA, Bare RL, McCullough DL. Systematic 5 region prostate biopsy is superior to sextant method for diagnosing carcinoma of the prostate. *J Urol* 1997; 157: 199-202; discussion 202-3.

18. National Health Committee. Prostate Cancer Screening in New Zealand. Report to the Ministry of Health (including attached Information Documents for Men and their Families and for the Health Care Practitioners). Ministry of Health: Wellington. 2004.

19. Humphrey PA. Prostate Pathology. American Society for Clinical Pathology: Chicago. pp 556, 2003.

20. Smith DS, Catalona WJ. Rate of change in serum prostate specific antigen levels as a method for prostate cancer detection. *J Urol* 1994; 152: 1163-7.

21. Vashi AR, Oesterling JE. Percent free prostate-specific antigen: entering a new era in the detection of prostate cancer. *Mayo Clinic Proc* 1997; 72: 337-44.

22. Hugosson J, Aus G, Lilja H, Lodding P, Pihl CG. Results of a randomized population-based study of biennial screening using prostate-specific antigen measurement to detect prostate carcinoma. *Cancer* 2004; 100: 1397-405.

23. Holmberg L, Bill-Axelson A, Helgesen F, Salo JO, Folmerz P, Haggman M, et al. A randomized trial comparing radical prostatectomy with watchful waiting in early prostate cancer. *N Engl J Med* 2002; 347: 781-9.

24. D'Amico AV, Chen MH, Roehl KA, Catalona WJ. Preoperative PSA velocity and the risk of death from prostate cancer after radical prostatectomy. *N Engl J Med* 2004; 351: 125-35.

25. Zhou M, Epstein JI. The reporting of prostate cancer on needle biopsy: prognostic and therapeutic implications and the utility of

diagnostic markers. *Pathology* 2003; 35: 472-9.

26. Steinberg DM, Sauvageot J, Pilantadosi S, Epstein JI. Correlation of prostate needle biopsy and radical prostatectomy Gleason grade in academic and community settings. *Am J Surg Pathol* 1997; 21: 566-76.

27. Gleason DF, Mellinger GT; Veterans Administration Cooperative Urological Research Group. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol* 1974; 111: 58-64.

28. Johansson JE, Andrén O, Andersson SO, Dickman PW, Holmberg L, Magnuson A, et al. Natural history of early localized prostate cancer. *JAMA* 2004; 291: 2713-9.

29. Spitz MR, Currier RD, Fueger JJ, Babaian RJ, Newell GR. Familial patterns of prostate cancer: a case-control analysis. *J Urol* 1991; 146: 1305-7.

Address for correspondence: Brett Delahunt, Department of Pathology and Molecular Medicine, Wellington School of Medicine and Health Sciences, PO Box 7343, Wellington South. E-mail bd@wnmeds.ac.nz.

Occupational Health and Safety Special Interest Group Meeting, April 3rd 2004, Heritage Hotel, Auckland.

The newly resurrected Occupational Health and Safety SIG meeting was held in conjunction with the Biochemistry SIG meeting. Registrations for both SIG meetings were combined to allow delegates to move between sessions - depending on the topic of interest. We expected up to 20 delegates per session and were pleasantly surprised and a bit caught out. Additional chairs had to be brought in as approximately 30 delegates attended each session.

The meeting was a great success with very interesting discussions, sharing of information and experience and most importantly established connections between delegates from various parts of New Zealand.

The combined opening plenary on stress management presented by Dr Jim McLeod placed everyone in the right frame of mind for the rest of the day. His presentation style delivered an underlining message that had a huge relevance for most of the audience. After that the two groups split into their respective disciplines for the rest of the day.

The papers presented within the Occupational Health and Safety SIG forum where both interesting and varied.

Dr Kenneth Whyte a Respiratory Physician at Auckland District Health Board spoke on the human sleep and circadian rhythms and implications for society including shift workers. His presentation was focused on the physiology and patterns of sleep.

Hadyn Olsen a Development Manager at Wave - Workplaces Against Violence in Employment had an excellent presentation on bullying in the workplace. Many questions and discussions raised by the delegates during and after the presentation.

Dr David Holland a Microbiologist at LabPlus spoke about biological hazards in laboratory. The presentation included discussion on existing and new potential pathogens such as SARS, risks of infection and how they can be minimised including information on what should be done if an exposure occurs. Well received presentation with valuable information.

Toni Morris, Quality Manager at Hutt Valley Laboratories spoke about an informal visit by OSH Inspector to their Histology department. The visit was a part of a nationwide ministerial inquiry into the management of certain hazardous substances.

Margaret Matson, Laboratory Scientist from LabPlus presented on the topic of eye accidents, preventative measures and practical solutions. Interesting data from the local laboratory survey collated and presented on eye accidents.

Natasha Saunders, Kendro Instruments New Zealand amazed delegates with pictures of 'centrifuge accidents' and information on forces involved when something goes wrong. Valuable information presented on what and what not to do when using centrifuges.

Murray Lewis, Nuplex Environmental spoke about the waste and what happens to it once it leaves the laboratory. Great information presented on legislative requirements, systems and methods in place to prevent accidents and pollution. It was nice to see what happens to waste we generate through pictures of various equipment and the waste plant.

Copies of the presentations have been posted on the NZIMLS website www.nzimls.org.nz

We would like to thank all the participants, delegates, and the sponsors: Abbott Diagnostics, Roche Diagnostics, Bayer Healthcare, Radiometer Copenhagen, Dade-Behring, Beckman Coulter and LabPLUS.

Branko Vidakovic. Occupational Health and Safety SIG convener

Factor VIII binding assay - one year on

David M Patterson, DipMLT, MNZIMLS, Senior Medical Laboratory Scientist, Haemostasis; Campbell R Sheen, BSc(Hons), PhD Student, Molecular Pathology; Canterbury Health Laboratories, Christchurch

As a follow-up to our previously published paper (1) we describe our experience during the first year using the Factor VIII (FVIII) binding assay. The assay is an ELISA based technique that measures the ability of von Willebrand Factor (vWF) to bind exogenous recombinant Factor VIII (rFVIII).

One of the two major functions of vWF is to act as a carrier for FVIII, thereby protecting it from proteolytic degradation. An abnormality in this carrier function of vWF leads to Type 2N von Willebrand Disease (vWD) which presents with a mild to moderate reduction in FVIII levels and is therefore difficult to distinguish from mild Haemophilia A. A FVIII binding assay is used to diagnose Type 2N vWD.

Over the past twelve months our laboratory has tested FVIII binding on fourteen patients. Of these, four individuals have been found to have significantly reduced FVIII binding capacity (Table 1).

Although the numbers of samples tested are reasonably small, it is significant that 29% have been found to be abnormal. This has an important clinical outcome, if the FVIII binding assay had not been performed these patients may have been incorrectly diagnosed as having mild Haemophilia A. A correct diagnosis of the autosomal recessively inherited vWD Type 2N allows for modification of therapy to accommodate for the shortened FVIII half-life in this disorder, resulting from the loss of stabilising effect of vWF-binding.

One of the patients (Patient B) with reduced FVIII binding has also been sequenced for known Type 2N von Willebrand mutations. This patient was found to be heterozygous for Arg854Gln, (Arg91Gln of the mature protein), which is the most commonly observed FVIII binding mutation (2). This patient was also found to be heterozygous for Arg924Gln (Arg161Gln of the mature protein), which is functionally normal in regards to FVIII binding but causes intracellular retention of the vWF protein (3). Therefore it seems that this patient is a compound heterozygote and further family studies are planned.

In conclusion, implementation of this assay has allowed detection of a significant number of patients with defective FVIII binding who otherwise may have been misdiagnosed as having Haemophilia A or Type 1 vWD. It is important to consider testing for FVIII binding in patients thought to have mild Haemophilia A with apparent autosomal inheritance or atypical bleeding symptoms and also in patients with Type 1 vWD where the FVIII level is disproportionately low.

References

1 Allan K, Patterson DM, Smith MP. Validation of a von Willebrand Factor - Factor VIII binding assay to characterise atypical phenotypes of apparent Haemophilia *A. N Z J Med Lab Science* 2003; 57: 12-5.

2 Mazurier C, Meyer D. Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand Disease - results of an international survey. On behalf of the Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost* 1996; 76: 270-4.

3 Berber E, James PD, Leggo, Collen N, Hagerdorn C, Cameron C; Lillicrap Association of Haemophilia Clinic Directors of Canada. Effect of the R924Q von Willebrand factor substitution on the expression of the von Willebrand disease phenotype (Abstract). *45th American Society of Hematology Meeting* December 6-9, 2003; San Diego, California, USA.

Address for correspondence: David Patterson, Canterbury Health Laboratories, PO Box 151, Christchurch. Email: David.Patterson@cdhb.govt.nz

Table 1. Abnormal FVIII binding results

Patient	FVIII (50 - 200%)	vWF:Ag[1] (50 - 200%)	vWF:RCo[2] (50 - 200%)	vWF:CBA[3] (50-250%)	FVIII binding (0.7 - 1.3)
A	29	62	80	66	0.1
B	13	31	35	30	0.2
C	18	75	77	85	0.3
D	24	91	na[4]	na[4]	<0.1

[1]von Willebrand factor antigen; [2]ristocetin co-factor activity; [3]collagen binding assay; [4]result not available

NZIMLS President's Address - 2004

2004 has been another busy year for the NZIMLS with the changes to the regulations governing the practise of MLS and the CPD programme being the main focuses. Over the past year there has been much interaction with the Medical Laboratory Technologist's Board (MLTB) over the "Scopes of Practise" for the profession, Registration of Laboratory Technicians, and the "Medical Laboratory Technician" scope of practise. The NZIMLS is supportive of the new directions of the regulations which have been introduced to provide greater safety for the public. Progress has not always been plain sailing however with concerns over the inclusion of Phlebotomists and Mortuary Technicians within the "Medical Laboratory Technician" scope of practise. The main concern has been over the classification of non-diagnostic activities as the performance of Medical Laboratory Science. The MLTB has decided to proceed with registration of these two groups as medical laboratory technicians. We feel that this is inappropriate and believe there is a strong case for inclusion of the non-diagnostic groups within a separate scope of practise. We have been informed that this could be re-considered at a later date.

This year Council agreed to stage a referendum on the continued Membership of the International Federation of Biomedical Laboratory Science (IFBLS). It was decided to make this move following continued doubt among some members as to the cost/benefits for membership and whether the financial commitment to the IFBLS might be better directed elsewhere. The results of the referendum were 41 in favour of withdrawal and 17 against. Council will decide in the upcoming year whether the Membership issue should be voted on at the 2005 AGM.

This year the NZIMLS moved toward joining the Allied Health Professions group. This is a group of representatives from most health and health-related professions who meet regularly to discuss matters relevant to members. At this time we are still awaiting official notification of acceptance. If this proceeds then the Institute will send a representative to the meetings to establish the benefits for the profession in the longer term.

Over the past year we have been working with AIMS and Statistics New Zealand towards updating the Australian and New Zealand Standard Classification of Occupations for the profession. The process has involved comparison of the educational and work experience requirements of both Scientists and Technicians in NZ and Australia against other professions. It is expected that we will be placed as MLS into the health professions group and the technicians into a group of associate professions. The purpose of the exercise is to provide a more uniform classification of personnel working in the profession between the two countries.

At the South Pacific Congress last year, the Institute signed an agreement for profit sharing with AIMS over future Congresses. The agreement was based on the number of delegates from the non-host country and was timely as it affected the 2004 SPC at Surfer's. The meeting performed well financially with the return to the NZIMLS a welcome financial injection in a year that usually impacts negatively on the accounts. Further financial details are provided in the Treasurer's report.

With the CPD programme now moving to a calendar year, the NZIMLS is investigating a move of the financial year to a calendar year. This will require a review of the rules with possible changes to accommodate the move. If the decision is made to proceed then this would allow the Institute to run a single billing system for both membership and the CPD programme.

The NZIMLS has over the past year sent representatives from Council or co-opted others to attend as many SIG meetings as possible. The main reason has been to promote the CPD programme and raise awareness among the profession of the changes to the MLS regulations that take effect from the 18th September 2004. Perhaps the greatest change in the upcoming year is the Medical Laboratory Science Board's requirement that all scientists/technologists must participate in a competency programme from 2005. With the NZIMLS CPD programme currently the sole approved programme, the Institute is expecting increased enrolments over the next 6-12 months. To meet the increased workload Council will move to provide greater resources for the programme. The "web-based" structure of the programme should help to control administrative costs however the appointment of a part-time programme manager and an increase in the hours of the Executive Officer is also planned. These together with support costs such as travel, website, office equipment, GST, stationary, and tax liability will mean a significant cost increase for the NZIMLS in the years ahead. Because of the difficulties in anticipating the likely income from the CPD programme Council has announced the likely cost of the programme over the last year to be between $56.00 and $200.00 year. The final cost of the programme has been set at $168.00 for individual non-members and $56.00 for individual NZIMLS members. Council has also introduced discounted rates for group membership because of the benefits of decreased administrative costs, dealing with larger groups. The cost of the programme has been set to prevent any negative financial impact on the Institute and to expedite the desire to make the programme available at a rate significantly lower for NZIMLS members. This approach is consistent with the costings for other services offered to members and non-members of the NZIMLS. This policy has not been received well by a few "non-member" practitioners.

In closing, Council believes that the CPD programme must not threaten the financial viability of the professional body. In NZ the overall percentage of MLS who belong to the NZIMLS is small and must increase if we are to have a strong professional body. It is Council's hope that the CPD programme will help to strengthen membership which will allow the Institute to continue to provide top-class continuing education and services to meet the future needs of the profession. In 2005 the Institute is poised to occupy an unheralded place in its 58 year history. Through the combined efforts of both previous and current Councils, I believe we are now well positioned for a period of growth that will benefit both the profession and the public of NZ.

Chris Kendrick
NZIMLS President

Cost and profit estimates for CPD programme - 3 years

Option 1
Members $56.00
Non-members $168.00

	2005			2006			2007		
Member status	numbers	price	Income	numbers	price	Income	numbers	price	Income
Members	400	$56.00	$22,400.00	1000	$56.00	$56,000.00	1200	$56.00	$67,200.00
Non-members	1020	$168.00	$171,360.00	420	$168.00	$70,560.00	220	$168.00	$36,960.00
Total	1420		$193,760.00	1420		$126,560.00	1420		$104,160.00
Costs									
Manager			$25,000.00			$27,500.00			$30,250.00
Secretarial			$10,000.00			$11,000.00			$12,100.00
Website			$4,000.00			$4,400.00			$4,840.00
Auditing			$5,000.00			$5,500.00			$6,050.00
Travel			$5,000.00			$5,500.00			$6,050.00
Stationary			$5,000.00			$5,500.00			$6,050.00
Equipment			$5,000.00			$5,500.00			$6,050.00
GST			$21,528.89			$14,062.22			$11,573.33
Total			$80,528.89			$78,962.22			$82,963.33
Net profit			**$113,231.11**			**$47,597.78**			**$21,196.67**

Conference Report

The 56th Annual Scientific Meeting of the NZIMLS was held at the Kingsgate Hotel in Hamilton from Wednesday 25th August to Friday27th August 2004. The workshops associated with the meeting were held on the Tuesday and Saturday. This was the fifth occasion that Hamilton had hosted the annual conference, the previous meetings having been in 1952, 1966, 1980 and 1994. As Hamilton is centrally located, the organising committee had expected a significant number of delegate registrations for the meeting, particularly day registrations. However, we were astonished when the total number of registrations exceeded 520 delegates plus exhibitors. This level of support for the meeting did result in some difficulties in locating sufficient accommodation, but in the end no one went without a bed. There was also considerable interest in the trade's exhibition and Fran had to be particularly creative in fitting the exhibitors into the available exhibition space. Unfortunately, lack of space meant that a small number of late applications to exhibit had to be declined.

The scientific sub-committee put together an outstanding scientific program with speakers of both international and national repute. The plenary sessions covered current concepts in such areas as gene therapy, transgenics and cloning, diagnosis of acute leukaemia and the New Zealand account of Meningococcal disease. The scientific sessions covered all of the traditional laboratory disciplines as well as the newer disciplines and specialist areas. For the first time at an NZIMLS conference there was a program for Molecular Biology and workshops for phlebotomy. The sessions on point of care testing and electronic reporting (HL7 messaging) attracted considerable interest, as did some of the multidisciplinary sessions encompassing topics as diverse as bioterrorism, drugs of abuse, the HPCA bill and uncertainty of measurement.

In the opening session of the meeting, Professor Tom Walker, otherwise known as the "Prof" from Maggie's Garden Show, delivered an informative and entertaining talk entitled "Soils, Plants, Animals and Health". He deftly related soil science to human nutrients and demonstrated a good understanding of the role of laboratory testing. He believes that soil is like a baby, you must keep its face clean, bottom dry and belly full. Also, like humans, soils go senile with age when they lose all their nutrients.

Robert Siebers delivered this year's TH Pullar memorial address in a presentation entitled "Outside Looking In". Rob gave an insightful reflection on the possible future of Medical Laboratory Science in New Zealand. In particular he gave consideration to the implications of an aging workforce.

Dr Barbara Bain from St Mary's Hospital, Imperial College of Medicine, London was the keynote speaker for the Haematology sessions. She is world renowned for her expertise in the area of blood and bone marrow morphology being the sole author of six Haematology textbooks and the joint author of two others. Dr Bain ran two superb full day workshops on red and white cell morphology as well as giving excellent state of the art presentations in the plenary sessions and haematology scientific program. Her sessions on the lymphoproliferative disorders were hugely informative, containing a wealth of practical information. She provided a number references to journals and websites, two useful ones being www.hmds.org.uk (haematology malignancy diagnostic service) and www.cancergenetics.com (FISH techniques and pictures). A current area of interest in haematology is the development of analyser decision rules. The International Consensus Group for Haematology Review has recently published a study of rules for haematology review. Dr Pat Barnes from One Barnes-Jewish Hospital, St Louis is on the consensus group, he presented the rules at our meeting and described how these will benefit haematology laboratories. The rules and data are available at www.islh.org.

A keynote speaker in the biochemistry program was Dr Graham Jones from St Vincent's Hospital, Sydney. Graham's current research interests revolve around the central theme of quality of laboratory results. His first presentation considered quantitative QC and questioned how sure laboratories are of the probability of their QC rules triggering and how good do the rule actually need to be. He believes that capable assays can have simple rules while poorly capable assays need more rules and QC run more frequently. His presentation is available for download at www.sydpath.stvincents.com.au .Consideration was also given to the effect of within subject biological variation. When analytical variation is one quarter of biological variation there is no need for analysis to perform any better. A database of biological variation is available at www.westgard.com/biodatabase1.htm. In his presentation on reference intervals, Graham made the point that we a not offering a good service to our customers as different laboratories frequently have different reference intervals for the same test and sometimes the same method. The AACB is doing some work in this area with a working party on common reference intervals. Other sessions of note in the biochemistry program included diabetes in pregnancy, fertility, and papers on the cardiac natriuretic peptides.

The general microbiology program provided a wide range of topics including antimicrobial resistance, *Helicobacter pylori, Staphylococcus aureus* bacteremia, *Chlamydia*, Verotoxigenic *E.coli*, infection control, and new concepts in microbiology.

All sessions were well attended, with many entertaining and informative presentations from both local speakers and guest speakers from overseas. Other aspects of microbiology were presented in topic specific sessions which included mycobacteria, mycology, public health, and virology. Richard Lumb from Adelaide, provided three very interesting presentations on mycobacteria. David Ellis, also from Adelaide presented in the mycology session as well as running a very successful mycology workshop.

A highlight of the conference was the plenary session on meningococcal disease, presented by Dr Graham Mills and Dr Diana Martin from ESR, detailing advances in early detection of meningococcal disease and the development of the new meningococcal vaccine.

The Point of Care Testing session was well supported by contributions from many of those from around the country involved in this area of testing. It provided an opportunity for participants to share their experiences in implementing the requirements of NZS/ISO 15189 as they pertain to POCT. A common thread running through the presentations was the need to gain the confidence and co-operation of the affected staff from throughout an organisation, in order to achieve a successful implementation of the standard. This buy-in also applies to the formulation of a Point of Care Policy, where agreement by all of the affected parties may not easily be reached and skillful negotiation, patience and persistence is required to achieve a consensus.

The scientific committee made a conscious effort to provide for the needs of those involved in preanalytical testing and phlebotomy. At the preanalytical session the concept of a QTA in specimen service was introduced. The draft specimen service (pre analysis) syllabus was presented for discussion. It was recommended that the syllabus

encompass the processes from after phlebotomy to analysis. The aim is to offer the examination for the first time next year. The course will comprise the core QTA syllabus, the specimen services specific section of the syllabus and a work book.. All have been developed and will be presented to Council for validation. Response to the concept, from both managers and staff in the audience, was excellent. Additionally, the formation of a Special Interest Group was promulgated. Lorna Gribble from Waikato Hospital has offered to act as the SIG convener during its first year of operation.

A phlebotomy workshop was held on the Saturday to allow as many phlebotomists to attend as possible. It attracted approximately 100 registrants with some coming from as far away as Timaru and Whangarei. The workshop began by honoring the top graduate from the 2003 inaugural Qualified Phlebotomy Technician (QPT) examination. A full program followed that was designed to both educate as well as extend both new and experienced phlebotomists. Topics covered included micro-capillary collecting, infection control, hepatitis, the HPCA act and the NZS/ISO 15189 standard as it applied to phlebotomists. The final session on examination techniques, entitled "how to pass the QPT" was appreciated by examination candidates and those contemplating sitting the phlebotomy qualification. Feedback from the workshop participants was extremely enthusiastic, confirming that phlebotomists greatly appreciated the opportunity provided for continuing education. There was a desire expressed for a phlebotomy workshop to be held on an annual basis.

Other workshops held in conjunction with conference this year included, publication and presentation skills, and examination setting and moderating, the latter workshop funded by Council and offered on a complimentary basis to current or intending QTA examiners and moderators. Both of these workshops were well attended and rated highly by participants.

Conferences are remembered as much for the social activities as the scientific program, and the consensus verdict from the party goers this year was of a highly successful and entertaining social program. Everyone entered into the spirit of the "P" party with many totally unrecognizable in their "P" costumes. "Professor" Richard Ward conducted the affairs of the evening and, with the help of his charming assistants, tested the trivia knowledge of those present. Skits from the Health Waikato Prozac Ducks were colorful, interestingly choreographed and on occasion left little to the imagination! The conference dinner on the Thursday evening was held at Vilagrads Winery and featured a sumptuous buffet dinner served under the grapevines. The band for the evening "The Sound Workshop" proved very popular with many taking to the dance floor from the first number played. The energy levels, from both the band and the revelers continued unabated until well after midnight when buses returned the merrymakers to their hotels.

The conference organising committee acknowledges the tremendous support provided by both the sponsors and exhibitors. Their backing helps to ensure the ongoing success of our annual scientific meeting.

Robin Allen, Conference Convener

Advertisers in this issue

Conference Pics

Correction

Bunker A. Is latent iron deficiency as benign as once thought? *N Z J Med Lab Sci* 2003;58:91-7.

Due to typesetting Tables in the Journal's usual format, Tables 1 and 2 from the above named article may be misleading to the reader. Below are the Tables as originally submitted. The Journal apologises for any misleading that may have arisen.

Table 1. Classic categorisation of iron deficiency

Stage	Pseudonyms used for stage
Stage I	Pre latent iron deficiency (pre LID)
	Early negative iron balance
	Storage iron depletion
Stage II	Latent iron deficiency (LID)
	Tissue depletion of iron without anaemia
	Iron deficient erythropoiesis
Stage III	Iron deficient anaemia (IDA)
	Manifest iron deficiency

Table 2. The expanded classification of the stages of iron deficiency

Stage I -	Pre latent iron deficiency (Pre LID)
	Early negative iron balance
	Storage iron depletion
Stage II	Latent iron deficiency (LID)
	Tissue depletion of iron without anaemia
	Iron deficient erythropoiesis
Stage IIIa	Iron deficiency erythropoiesis with Hb in the reference range which increases with supplementation
	Relative / functional IDA
Stage IIIb	Iron deficient anaemia (IDA)
	Manifest iron deficiency

Career Transplant?

Have you ever considered working in the UK? Corinth Healthcare is one of the UK's largest healthcare agencies with offices all over the world.

- Thousands of jobs across the UK
- Exceptional rates of pay
- Help with visas and work permits
- Consultant dedicated to you

For over 35 years, Corinth has been finding the best jobs for the right people across the UK, from exciting and vibrant cities to the peace and tranquility of the countryside.

Find out more about working in the UK, call now on

0800 446 276

ukjobs@corinthhealthcare.com
www.corinthhealthcare.com

corinth laboratory

CSCI Registered

Committed to equal opportunities

Abstracts from the ASM of the NZIMLS, Hamilton, August 2004

Acute leukaemia-from phenotype to genotype

Bain B. St Mary's Hospital, London, UK

Leukaemia was initially recognized, in the mid 19th century, on the basis of its clinical and pathological phenotype. In 1845 John Hughes Bennett in Edinburgh and, six weeks later, Rudolf Virchow in Berlin described instances of this illness, on the basis of autopsy examination including microscopic examination of the blood. The diseases they described, probably chronic myeloid and chronic lymphocytic leukaemia respectively, were characterized by 'Weisses Blut' (white blood), the term 'Leukhemia' being first used by Virchow several years later. Within a decade Nikolaus Friedreich in Wurzburg had described a case of acute leukaemia, probably T-lineage acute lymphoblastic leukaemia. The next one and a half centuries saw great advances in the phenotypic diagnosis of leukaemia attributable to improved microscopes, better stains (initially based on aniline dyes), examination of the bone marrow (initially after death but subsequently during life), cytochemical stains and, rather later, immunophenotyping. Leukaemias were separated initially into a small number of phenotypic groups but, as characteristic cytological, cytochemical and immunophenotypic features of various leukaemias were recognized, the number of specific disease entities that could be recognized multiplied. The various FAB classifications of leukaemia can be seen as the culmination of our attempts to characterize leukaemia phenotypically.

While the FAB group were working on the precise phenotypic diagnosis and categorization of leukaemia, attention was also turning to genotypic features. Attempts to systematize this knowledge, initially on the basis of cytogenetic features, were made by the various MIC working parties, which proposed a number of MIC (Morphologic-Immunologic-Cytogenetic) classifications. Later, with advances in molecular genetic techniques it was recognized that subtypes of leukaemia could also have characteristic molecular features and the idea of leukaemia as an acquired genetic disease was born. Cytogenetic abnormalities had already been recognized as informative with regard to aetiology and prognosis. Now it became apparent that the cytogenetic abnormalities were only important because they reflected the presence of a specific molecular genetic abnormality or abnormalities. Furthermore, equally important molecular genetic abnormalities could be present without there being any apparent relevant abnormality in the chromosomes viewed microscopically. A MIC-M (Morphological-Immunological-Cytogenetic-Molecular genetic) classification, incorporating this new knowledge, was proposed; although the expanded acronym did not become popular, many haematologists were following this approach.

The FAB classification of acute leukaemia started as a morphological (or phenotypic) classification. Morphology was later supplemented by immunophenotyping to permit the definitive diagnosis of acute lymphoblastic leukaemia (ALL) and the FAB categories of M0 and M7 AML. Diagnostic advances that have occurred since then have been in the fields of cytogenetics and molecular genetics and this has led to increasingly sophisticated diagnosis. Acute leukaemia diagnosis now requires (i) clinical history and physical examination (ii) blood film and count (iii) bone marrow aspirate and cytogenetic analysis (iv) immunophenotyping for those cases that are not obviously myeloid (v) molecular genetic analysis, as a supplement to cytogenetic analysis, to identify good and bad prognosis subgroups that require specific management. The World Health organization (WHO) classification recognizes these recent advances to some extent, though perhaps not as much as one would wish.

The WHO classification of AML is a hierarchical classification that first identifies cases with previous exposure to potentially leukaemogenic therapy. Remaining patients are then assigned, when appropriate, to four specific cytogenetic/molecular genetic categories. They are:

- AML with t(8;21)(q22;q22) and AML1-ETO fusion
- AML with abnormal bone marrow eosinophils with inv(16)(p13q22) or t(16;16)(p13;q22) and CBFB-MYH11 fusion
- Acute promyelocytic leukaemia with t(15;17)(q22;q12) and variants
- AML with 11q23 (MLL) abnormalities.

After the assigning of cases to these two categories other cases, even those with other recognized recurring cytogenetic or molecular genetic abnormalities, are assigned to categories that are purely morphological, specifically:

- AML with multilineage dysplasia
- AML, not otherwise categorized (with subcategories resembling the FAB classification).

The WHO classification of ALL is immunological rather than morphological-precursor B and precursor T. Cases with a mature B phenotype (correlating with the FAB L3 category) are classified as non-Hodgkin's lymphoma.

Cancer, including leukaemia, is viewed as being the result of an accumulation of multiple mutations in a cell that ultimately undergoes a malignant transformation, leading to a disease phenotype-the multi-hit hypothesis of cancer causation. In some subtypes of acute leukaemia, there have clearly been a considerable number of cytogenetic and molecular genetic events, conforming to this hypothesis. In other subtypes there may have been only a small number of such events, perhaps as few as two. Two WHO categories can be seen as having cases that have mainly had a considerable number of genetic events in the leukaemic clone, specifically therapy-related AML and AML with multilineage dysplasia whereas the four specific cytogenetic/molecular genetic categories include many cases that are likely to have had fewer genetic events.

The purpose of any classification of AML is to recognize biological entities, in order to both to advance scientific knowledge and improve the treatment of patients. The possibility of developing drugs that might block the transcription or translation of a specific DNA sequence or might inhibit a specific oncogenic protein, as is already occurring in chronic myeloproliferative disorders, is one of the major justifications for a genotypic approach to the classification of AML. Although the discovery of the remarkable efficacy of all-trans-retinoic acid (ATRA) in M3 and M3 variant AML was serendipitous, its effect in this type of leukaemia, characterized by a PML-RARA fusion gene, is explicable in molecular terms. The developments in the chronic myeloid leukaemias suggest that in future effective drugs for specific subtypes of AML might be deliberately designed. The drive to do this would clearly be greatest for those molecular events which recur with a high frequency, specifically AML1-ETO in association with t(8;21)(q22;q22), CBFB-

MYH11 in association with inv(16)(p13q22) or t(16;16)(p13;q22) and in frame internal tandem duplications of FLT3. FLT3 encodes a receptor tyrosine kinase of the PDGFR superfamily and is the receptor for flt3 ligand. It appears a particularly important target for new therapies since mutations are common and are associated with a poor prognosis. Approximately 20% of adult patients with acute myeloid leukaemia have such mutations; they occur across all FAB subtypes but particularly in FAB M5b. FLT3 inhibitors for the potential treatment of AML are already under development.

Conclusions: acute leukaemia diagnosis must be founded in a good clinical assessment and traditional morphology but this must be supplemented by newer techniques to further specify the precise type of acute leukaemia and thus both advance scientific knowledge and improve patient management.

Molecular aspects of bcl-2 expression in human B cells

G Lang, Institute of Cancer Research, London, UK

Elevated expression of bcl-2 post t(14;18) translocation increases the lifespan of the cell and thus promotes the development of follicular lymphoma. We have investigated the protein binding sites in a DNase I hypersensitive site associated with Bcl-2 gene expression in human B cells. Electrophoretic mobility shift assays (EMSAs) with extracts from the REH B cell line reveal three B-myb binding sites in this sequence. The levels of B-myb and Bcl-2 protein and EMSA activity are correlated across a range of B cell lines representing different stages of B cell development. Antisense B-myb treatment of REH cells down-regulates EMSA activity and Bcl-2 protein and leads to the apoptosis of REH cells. Expression of B-myb in the Bcl-2 non-expressing RPMI 8226 B cell line leads to the appearance of EMSA activity and Bcl-2 protein. DNA containing each of the three myb binding sites, separately linked to the 3'-end of a reporter gene, enhance reporter gene transcription in REH cells; mutation of the myb binding site destroys this activity. The same constructs fail to stimulate transcription in RPMI 8226 cells, but co-transfection with a myb expression vector activates transcription to the same level as observed in REH cells. A construct containing the Bcl-2 gene promoter region through exon-2, linked to a reporter gene, enhances reporter gene transcription in REH cells; deletion of the three myb binding sites ablates this activity. Our results suggest that the DNase I hypersensitive site represents a Bcl-2 gene enhancer responding to B-myb in human B cells.

Thrombosis risk factors: ZPI - the new kid on the block

N Van De Water. LabPlus, Auckland Hospital

The coagulation system is a dynamic process requiring both activators and inhibitors to maintain normal haemostasis. Disturbance of this balance due to a deficiency of one of the inhibitors can predispose to thrombosis. Genetic risk factors for thrombosis such as factor V Leiden, a deficiency of protein C, protein S and antithrombin are well established but only account for about 20-30% of thrombotic events. Other environmental or as yet unidentified inherited risk factors are clearly involved. Protein Z dependent protease inhibitor (ZPI) is a serpin which inhibits coagulation factors Xa and XIa. The precise physiological significance of ZPI in the control of haemostasis is unknown although a deficiency of ZPI may be predicted to alter this balance. In a study of thrombosis patients and age matched controls the coding region of the ZPI gene was screened for mutations. We identified 16 mutations/polymorphisms within the coding region of ZPI including two mutations, which generated stop codons at residues R67 and W303. These nonsense mutations within the ZPI gene were observed in 4.4% of thrombosis patients (n=250) compared with 0.8% of controls (n=250). The difference in distribution of stop codon mutations between thrombosis patients and controls was significant ($p = 0.02$) with an odds ratio = 5.7 (95%CI, 1.25 - 26.0). Our results suggest an association between ZPI deficiency and venous thrombosis and we propose that ZPI deficiency is potentially a new form of thrombophilia.

Direct thrombin inhibitors - laboratory monitoring

R M C Allen. Waikato Hospital, Hamilton

In patients with venous thromboembolism, anticoagulation with heparin is well established and is the standard initial treatment. Unfractionated heparin (UFH) is administered intravenously and requires dose adjustment, usually based on the activated partial thromboplastin time (aPTT). Low molecular weight heparins (LMWHs) have been introduced as equally effective and safe alternatives to UFH in the treatment of deep-vein thrombosis and pulmonary embolism. LMWHs differ from UFH in that they have a greater anti-factor Xa to anti-IIa activity, greater bioavailability, longer plasma half-life, a more predictable anticoagulant response, and less adverse events such as heparin-induced thrombocytopenia. Recently, numerous new substances with directly acting inhibitory actions to one or more binding sites of thrombin have been discovered or systematically developed by analogue design. These direct thombin inhibitors (DTIs) have a small molecule size and can effectively inhibit clot bound thrombin. Since the introduction of the first DTIs, monitoring of the direct inhibitory effects has been addressed as an issue. While extension of the aPTT to DTI monitoring has been promulgated, the method is now considered to be unsatisfactory due to poor linearity and reproducibility.

Bone banking in New Zealand - future direction

S Ghosh. New Zealand Blood Service, Waikato, Hamilton

In New Zealand there are 13 bone banks and an unknown number of facilities collecting and storing bones in a mostly unaccredited manner. Though most bone banks follow one guideline or another, there is no consistent practice. Collection, testing and storage practices vary from bone bank to bone bank. Almost all of the bones collected are femoral heads from live donors. Very little effort has been made to provide specialized products such as long bones and tendons, although demand for such products is increasing. There is an urgent need to address the current situation with a single standard across the country and implement proper control and audit of the process including a plan to meet the changing demand.

POCT connectivity, the Auckland experience

C Rouse. LabPlus, Auckland Hospital, Auckland

The Public hospital system in Central Auckland has undergone some major structural changes in the past 10 years, creating from the original four individual hospitals of National Women's, Greenlane,

Auckland and Princess Mary Hospitals, one super hospital, Auckland City Hospital. The Focus of the new hospital which opened late in 2003, has been towards the use of technology, with Laboratory and Radiology results being viewed and signed off on line, and all patient notes being available securely online. The Laboratory has recently been involved in a tender process to replace the Hospital's aging fleet of blood gas analysers. As part of this process, we have also undertaken to implement a connectivity package to help monitor and maintain these new instruments, particularly those located outside of the Laboratory. Currently, ACH has a total of 14 blood gas analysers used throughout the organisation, 3 are based in the Laboratory on two sites the remaining 11 are based in theatres, neonatal, paediatric, emergency and cardiac critical-care wards. Five of these ward analysers are connected to the Radiometer radiance connectivity package with plans for more to follow. The system is not limited to Radiometer based systems alone. This has lead to a paperless maintenance system and centralised warnings of problems in wards (some times even before the ward is aware of the problem) and remote trouble shooting. We have also set up an interface with the Radiance Blood gas database to allow transfer of all ward results into the central laboratory result system for easy storage and retrieval. This model will also be applied to the hospital's POCT Glucose monitors when they are replaced later this year.

Remote thrombelastograph network

G Devenie. LabPlus, Auckland Hospital, Auckland

The Thrombelastograph (TEG) is a whole blood clotting analyser that detects the elastic properties of the clot as it develops. Auckland District Health Board (ADHB) has six TEGs; two are in level 8 general theatres, two are in level 4 cardiac theatres, one is in cardiac ICU and one in the haematology laboratory. ADHB was selected as a BETA testing site to develop the new software. This was achieved by a coordinated effort from ADHB's IT department, surgical services on Level 8, haematology's POCT technical specialist, the NZ distributor, Medtel NZ and the manufacturer, Haemoscope in the USA. The successful utility of a POCT analyser requires the results to be with the clinician as soon as they are available. This was achieved by installing the latest TEG Enabled and TEG Remote software. Nineteen computers in theatres and ten computers in medical units now have the TEG Remote software to view tracings as they develop. Prior to implementation of the TEG Remote software, the technician would have done multiple trips to get printouts of the tracings. Improvement has been noted in the turnaround time for clinical decisions.

Training and competencies

L Clarke. Waikato Hospital, Hamilton

Many of us train others in the way that we have been trained without giving much thought as to whether it is the most effective way to carry out the task. The learner's needs may not be mine, nor may their preferences for learning style be accommodated. The aim of this presentation is too heighten awareness to the pitfalls for trainers and offer some practical tips.

- Set objectives and establish performance standards.
- Use the formula (what they need to be able to do) - (what they can already do) = instruction.
- Perform a task analysis to clarify each step that you are going to teach them and write yourself a checklist.
- Establish control signals to enable the learner to monitor their performance.
- Prepare the environment and the trainee prior to the training session.
- Provide a context, communicate clearly and check understanding.
- Use questions throughout to establish how much they know already, to focus their attention and to check whether they have understood your instruction.
- Give feedback that tells the trainee how their performance measures up to the established performance standards.

Conclusions

On the job training allows us to customize our training sessions to meet the individuals needs and involve them in the process, thus making our training more effective.

References

1. Mager RF. Making Instruction Work (2nd ed.). 1997. CEP Press, Atlanta, USA

2. Ramsey P, Franklin T, Ramsey D. On The Job Learning, Creating Productive Work Environments. 2000 Dunmore Press Ltd, Palmerston North, New Zealand

An unexpected diagnosis

S M Holland. LabPlus, Auckland Hospital, Auckland

Haemoglobin Barts hydrops fetalis is a syndrome consequent on an absence of all four alpha genes and a consequent total lack of all alpha globin chain synthesis. The result is severe anamia and hypoalbumin causing stillbirth or early neonatal death of a hydropic foetus. This is a case study of a male Chinese baby that was born on 22/02/04 at National Womens Hospital by emergency caesarean and was severely flat and hypoxic. A cord sample was taken before the baby was transfused. On this sample we performed a full blood count and haemoglobin electrophoresis which showed the presence of mainly haemoglobin Barts. Subsequent testing of the mother and partner showed a thalassemic blood picture with low MCH and MCV values and a normal haemoglobin electrophoresis. Haemoglobin H was found in both. This occurrence is becoming more relevant as the population of South East Asians is increasing in Auckland. We have to be aware of the importance of careful antenatal testing and the need for DNA testing if the partner has abnormal indices.

Process solutions for haematology. One laboratory's journey toward total lab automation

Pat Barnes. One Barnes-Jewish Hospital, St Louis, USA

Between 1985 and 1990 our laboratory experienced a tremendous increase in workload. In just 5 short years we saw testing volumes increase by over 68%. At the same time healthcare reimbursement in the US has undergoing radical changes. Each year what we were receiving in payment from the federal government and other insurers was steadily being decreased. Managed care and fixed payments per patient (capitation) was becoming the rule. With these forces in play our laboratory began in 1990 to seek more effective efficient ways to maintain high quality laboratory services with shrinking operating budgets.

Between 1990 and 1995 we directed our efforts toward non-automated process improvements including: basic instrumentation; consolidation of laboratory services; staff cross training; workstation

redesign; auto-verification of test results; and new differential rules.

Between 1995 and 2000 we focused on automated solutions including the introduction in 1997 of the first fully robotic haematology system in the world. We also began to develop plans for the full automation of coagulation, which finally occurred in 2001. Since 2001 we have upgraded our automation with the introduction of third generation robotics such as the LH-1500. We also continue to look at future improvements in coagulation automation, where we have just completed an evaluation of the ACL-TOP as a potential replacement of our current coagulation analyzers on our robot line.

Haemovigilance - what's going on?

S Benson. New Zealand Blood Service, Auckland

The New Zealand Blood Service has statutory responsibility for the safety of blood transfusion in New Zealand and is currently in the process of developing a 'National Haemovigilance Programme'. The provision of safe blood transfusion therapy is a basic requirement of advanced medical care. Blood and the components or products derived from it are inherently safe with an extremely low risk of transmission of infectious agents such as HIV, HBV or HCV. However, the process of blood transfusion is not as safe and carries a considerably higher risk of adverse consequences. Although there are a number of possible adverse consequences of transfusion, the majority of reported events are due to procedural errors that result in patients being transfused with a blood component or plasma product which does not meet appropriate requirements or which was intended for another patient. Thankfully the patient outcomes of adverse events are generally minor, however, there are still avoidable deaths or major morbidity associated with transfusion. National haemovigilance programmes have been set up in a number of countries with broadly the same goals of collecting and collating data on adverse events associated with transfusion of blood and blood components. In collecting (and reporting) this data the primary outcome is improving transfusion safety.

Safety - current status

P Flanagan. New Zealand Blood Service, Auckland

The safety record of blood components and products currently available for transfusion in New Zealand is excellent and compares favourably with the international position. The introduction of Nucleic Acid Testing for HIV and Hepatitis has dramatically reduced the likelihood of transmission of these agents; current estimates indicate this is less than 1 in 2 million components transfused. A number of concerns remain, however. Each year NZBS receives 1-2 reports of possible transmission of Hepatitis B by tested blood. Two possible approaches to risk reduction are currently being investigated. These are Hepatitis B core antibody testing and the use of HBV DNA screening. There is increasing recognition internationally of the risks associated with bacterial contamination of blood component, particularly platelets. The risk can be reduced by the use of two stage skin cleansing and the use of diversion systems incorporated into blood packs. NZBS has initiated a pilot study to investigate the level of contamination seen in platelet concentrates. This data will assist in deciding whether current approaches to risk reduction in this area are adequate or whether a move to formal pre-release testing of platelet concentrates is appropriate. Thirdly, the report in December 2004 of a possible transmission of vCJD by component transfusion in the UK reinforces the importance of appropriate precautionary measures in this area. Increasingly focus is directed to the balance between cost, safety and sufficiency. The real challenge for blood services in the 21st century is to ensure that this is effectively managed.

Hemoximeter: application in the cardiac cathlab

K A Timmins. Waikato Hospital, Hamilton

The cardiac cathlab at Waikato hospital uses a hemoximeter machine to analyse oxygen saturations in blood samples taken from various sites in the heart. The results help in the diagnosis and treatment of patients with shunts caused by atrial or ventricular septal defects. A Swan Ganz catheter is used to gain access to the heart through the femoral artery. Dry lithium heparinised syringes are used by the cardiologist to collect samples. They are analysed by a cardiac technologist who relays the results directly to the cardiologist. The blood samples are put through the hemoximeter where they are hemolysed by ultrasound and measured at six wavelengths. Oxygen saturation (%) is calculated from the concentration of oxyhemoglobin and deoxyhemoglobin. A decrease in the level may indicate an impaired ability for the oxygen capacity of hemoglobin. An increase in the oxygen content in the right-sided heart chambers beyond the normal variation is termed a 'step up'. This suggests a left to right shunt where oxygenated blood from the left side of the heart is mixing with deoxygenated blood in the right side of the heart. The treatment of these septal defects depends on the size of the defect, it's location and the patient's symptoms and may include medication or surgery.

Antimicrobial resistance - the war we cannot win

S Jaksic. Pathology Associates

Since the introduction of sulfonamides almost 70 years ago, there has been an ever-escalating battle between man's ability to find and create new antimicrobial agents and the ability of microorganisms to find mechanisms to circumvent the action of these agents. After years of heavy use of antimicrobials to treat billions of patients, we still face the increasing challenge of resistance. Recent scientific breakthroughs have enlightened our knowledge of the world of microorganisms and allow us to better understand the resistance as a part of their life style, ie. why the challenge will never end. The phenomenon of the emergence and spread of antimicrobial resistance simply reflects the versatility and apparent ingenuity of microorganisms to adapt to their general environment. For patients and healthcare workers, resistance to antimicrobials creates many problems -it leads to the use of more broad spectrum agents, which are more expensive and potentially more toxic and often also to costly infection control measures. In the worst possible scenario, there may no longer be an agent to which to an infecting organism is susceptible (or, in the developing world, there may not be an affordable one). There is currently no foreseeable end in sight to the development of antimicrobial resistance while, at the same time, alternative measures such as vaccines or immmunotherapies do not offer an immediate end to the need of antimicrobial therapies. Thus, continued searches for new agents, combined with their judicious use and with efforts to identify and contain new resistance traits, as well as strategies to prevent infections, are needed.

LOINC codes and eLab

S Chan. Diagnostic Laboratory, Wanganui

Medical laboratories in NZ have been using the HL7 (Health Level Seven) standards since 1994 to send their results electronically to patient management systems (PMS) of medical practitioners. However, pathology laboratories identify tests in these messages by their internal and idiosyncratic codes. Thus, PMS cannot fully 'understand' and properly file the results they receive unless they either adopt the producer's laboratory codes (which is impossible if they receive results from multiple sources) or invest in the work to map producer's codes to their internal codes. LOINC (Logical Observation Identifier Names and Codes) provides universal identifiers for laboratory and other clinical observations. The purpose of LOINC is to facilitate the exchange and pooling of results or vital signs for clinical care, outcomes management, and research. LOINC has been endorsed in USA by HL7, professional bodies, government institutions and commercial organisations; and has also been adopted by other countries. In 1998, medical laboratories in NZ began to show interest in LOINC. A lot of work has been done through the Association of Community Laboratories (ACL) and the NZ HL7 User Group (NZHUG). In 2004, Health Information Standards Organisation (HISO) accepted a proposal by NZHUG to formally adopt LOINC for use in NZ. Considerable success has been achieved and further potential has been revealed for using LOINC in electronic laboratory ordering and electronic clinical documents. However, there are still issues which require attention by stakeholders such as the government, software vendors and health professionals before national implementation is possible.

Bacterial contamination of the Waikato River - an environmental detective story

D Hood, Public health Unit, Waikato Hospital;
I Liddell, NZAP Ltd, Cambridge;
B Campbell, Environment Waikato, Hamilton

In March 2002, routine microbiological monitoring of the Waikato River revealed sustained high levels of "E. coli" which could not be explained by rainfall and agricultural run off. Warm weather and high recreational use of the river meant human health was at risk. Environment Waikato's investigations indicated a point source rather than the generalised agricultural impact that was initially assumed to be the cause. The source proved to be a consented and well-monitored discharge from a dairy factory, where a series of circumstances had coincided to allow massive bacterial overgrowth go unrecognised, probably for many years. The pollution was controlled immediately and the river quality improved within 24 hours. With the cause elucidated, a potentially widespread environmental hazard was prevented. Subsequent investigations indicated there was minimal health risk posed by the dairy factory discharge.

Introduction

Environment Waikato undertakes regular monthly sampling of 110 surface water sites, 10 of these are along the Waikato River. Testing for bacterial quality using Escherichia coli (E coli) is part of that monthly monitoring. In addition Environment Waikato carries out summer surveys of bacterial quality in contact recreation waters.

In March 2002 both Environment Waikato and the Waikato District Health Board (WDHB) found higher than expected E coli levels in the Waikato River near Hamilton. By late March - early April 2002 the WDHB had found three consecutive samples had E coli levels greater than 2400 cfu/100mls in Hamilton. This was well above the Recreational Water Guidelines action level of 410 E coli per 100ml, and was of high concern to both Environment Waikato and WDHB. These concerns triggered a public health warning by Dell Hood as Medical Officer of Health, and an intensive search by Environment Waikato to locate the source.

Source investigation

The E coli increase was known to start between Cambridge and Hamilton. Environment Waikato water scientists estimated that the mass bacterial increase would be equivalent to a discharge of about 50 L/s of raw sewage. This caused consternation, as the largest known sewage source in the area was the Cambridge treated sewage; however this point source had a smaller flow and was well treated, and testing quickly confirmed that it was not the source of contamination. Environment Waikato undertook three detailed river surveys in this area on 7th, 18th and 30th April 2002, as well as several tributaries, and inspected various consented discharges which had animal wastes in them.

The river survey results indicated that E coli levels greatly increased at a point 6 kilometres below Cambridge. There were two point sources near there - a private secondary school and Fonterra Hautapu. The Fonterra discharge was sampled, but as it looked like drinking water there was no expectation that this would be the source. In contrast, the school sewage ponds were found to be leaking and were an obvious source of faecal bacteria into the river, although the scale was too small to account for the known E coli levels in the river.

However, on 29 April 2002 the dairy factory was found to be the source of the river contamination, with 47 million E coli per 100mls in the factory discharge.

The nature of the bacterial discharge

Once the high E coli levels were brought to Fonterra' attention, the company reacted promptly, shutting down the discharge to the river within 24 hours. The discharge was diverted to the Fonterra land treatment system and was not resumed until chlorine dosing was installed.

The high E coli levels in the Fonterra effluent were a surprise to both Fonterra and Environment Waikato. The company had been granted a renewal consent to discharge to the Waikato River in February 2000, and E coli levels in the effluent had been assumed to be low. There was no sewage component in the effluent, nor known source of infection. The wastewater consisted of various clean streams of condensate and reverse osmosis permeates, from food processing.

Fonterra commissioned reports to find the source of infection within the factory, as well as long-term disinfection options. These reports confirmed that there was no known source of sewage, and that the majority of the waste streams had nil E coli most of the time. Occasionally the waste streams had low numbers of E coli (<10 per 100mls). However, storage of the relatively clean wastewater in a waste silo, prior to discharge to the river, allowed faecal coliform bacteria to build to very high levels. A further 3-4 hour travel time down an enclosed pipeline to the river, allowed further bacterial growth. The predominant bacteria was E coli, although other bacteria found in high numbers were *Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae, Serratia rubidaea, Citrobacter* and *Pseudomonas*. Testing for true sewage pathogens such as *Salmonella, Campylobacter, E coli O157* or *Legionella* showed these were not present.

As part of its investigation into the incident, Environment Waikato found that a similar incident occurred at a dairy factory at Stirling, Otago, in February 2001. At that site the peak faecal coliform level was 1200 million per 100mls!

By May 2002 Environment Waikato compliance officers considered that no legal action should be taken against Fonterra for the river contamination, since:

- There was no evidence that Fonterra could have easily foreseen or have reasonably be expected to know that their river discharge was heavily contaminated by faecal coliforms;
- External investigations indicated that the factory food processing and equipment cleaning processes were normal for the industry, if not better;
- The company took prompt and effective action once they were aware of the problem;
- The company fully co-operated with subsequent investigations.
- Other than the E coli problem, the company had a high level of compliance with its other consents.

EW Councillors accepted the recommendation that no legal be taken.

Discussion

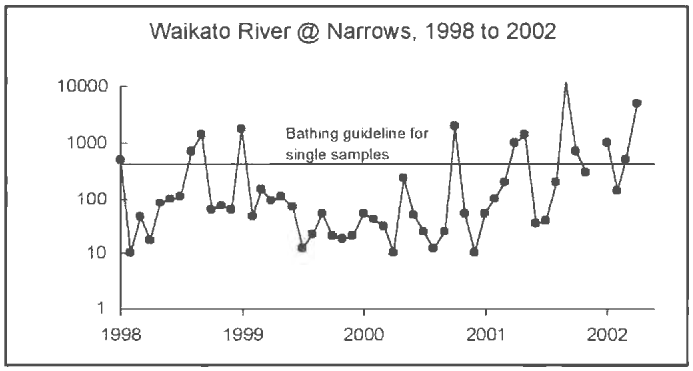
The high E coli concentrations found by Environment Waikato and WDHB in March 2002 eventually turned out to be a false alarm, but this was not known at the time. The value of E coli as an indicator of water quality, with respect to recreational use and public health risk, is that it is a relatively cheap laboratory test, and normally it accurately assesses how much sewage is present in the water. With the preliminary information that the WDHB had in March 2002, I consider it was a correct decision by Dell Hood to publicise the health risks posed by the Waikato River, and to take urgent action.

However, the high numbers of faecal coliforms found in the Fonterra discharge were not of human sewage origin. It is possible that the faecal bacteria were of general environmental origin, perhaps present in the air within the silo, or perhaps present within the silo for many years as a surface film. The warm silo, and the nutrients present in the wastewater (traces of minerals, high carbohydrate), enabled specific faecal coliform bacteria to proliferate under optimal growth conditions. The bacterial species identified in the silo effluent were considered to pose minimal risk to public health.

Personal communication with Andrea Donnison, AgResearch Ruakura, in 2002 indicated that high levels of E coli and Klebsiella may be found in some wood processing waste ponds, due to the specific nutrients present in those ponds.

Not withstanding this low risk, the high levels of faecal bacteria from Fonterra in the river were not acceptable to either EW or WDHB because they potentially masked real sewage inputs. EW uses the region-wide river monitoring data to identify long-term water quality trends, as well as more localised effects. From time to time, this regular monthly monitoring indicates unusual contamination, and EW takes enforcement action against unauthorised point discharges when located.

EW monitoring of the Waikato River just upstream of Hamilton is shown in figure 1 below. It shows that in general the water met bathing water guidelines, but also showed an apparent deterioration in 2001-2002.



Data from B Vant, Environment Waikato, 2002

In conclusion, the March-April 2002 Waikato River contamination incident raised some interesting problems for authorities. The high faecal coliform levels in the Waikato River, at a time of high recreational use, led to public health warnings by the WDHB. It was initially assumed by the WDHB and Environment Waikato that the source was either sewage or other warm-blooded animal wastes, and that this would pose a real threat to public health. Later, the bacterial contamination was found to be from a relatively harmless source, and the public health warnings were withdrawn.

While the E coli and faecal coliform lab tests are useful indicators of faecal contamination of surface water, they are just indicators. During incidents such as outlined here, authorities must confirm the source and the public health risks as soon as possible. There must be effective liaison between public authorities to enable this to happen.

Spa pools and Mycobacterium avium complex - lung disorders in immunocompetent adults

R Lumb. Institute of Medical and Veterinary Science, Adelaide, Australia

Outdoor spas are characterised by having stored water heated to approximately 35°C, greater bather load to water volume ratio, vigorous aeration of the water body, and elevated organic contaminant loading. The warm, aerated water provides an ideal environment for the rapid growth of microorganisms. Previously healthy adults have developed Mycobacterium avium complex (MAC) - lung disorders associated with chronic exposure to spa waters. A combination of pulse-field gel electrophoresis and PCR-amplification of genomic sequences located between the repetitive elements IS1245 and IS1311 was used to examine the relatedness of MAC isolates from clinical and environmental sources. Genotyping demonstrated a strong relationship between MAC isolates obtained from patients and their respective spa water and in three cases, rainwater used to supply the spa. Persistence of the disease-associated genotype was demonstrated in one spa pool for over five months until repeated treatments with greater than 10 milligram per litre of chlorine for one-hour intervals eliminated M. avium complex from the spa pool. The nature of lung disorders, either hypersensitivity pneumonitis, or true infection, or both, may be difficult to establish, and may represent a continuum between the two conditions. In all cases, inadequate spa pool maintenance was responsible for allowing large numbers of MAC to arise in the spa water. Collaboration of clinicians, laboratory and environmental health officials was required to achieve a successful outcome for all cases.

Agricultural and medical opportunities from livestock cloning and transgenics

D Wells. AgResearch, Hamilton

Farmers have been modifying the genetic composition of animals (and plants) using selective breeding for millennia. The more recent technologies of cloning and genetic modification provide the potential to enhance this process further, providing new agricultural and medical opportunities. In mammals, the technique of nuclear transfer enables the reconstruction of a cloned embryo from a donor nucleus and a recipient oocyte cytoplasm. Upon exposure to factors present within the oocyte cytoplasm, the nucleus from a differentiated somatic cell (perhaps from an adult animal) can be "reprogrammed". If the normal sequence of gene expression occurs during embryonic and

fetal development (following embryo transfer to a surrogate female), a cloned animal may ultimately be born with the same nuclear genetics as the original donor. Whilst possible, this process is inefficient and highly prone to epigenetic errors. The dysregulation of gene expression leads to the continual loss of clones throughout pregnancy and the peri-natal period. Currently, in cattle, only around 10% of cloned embryos transferred result in viable offspring. The high mortality raises serious animal welfare concerns that limit the present acceptability and applicability of the technology. With increased understanding of epigenetic reprogramming, however, it is expected that the present efficiencies will be improved. As with all new reproductive technologies, any long-term consequences of cloning need to be comprehensively evaluated. Whilst there are reports of apparently healthy clones in adulthood, other studies, especially in the mouse, have shown cases of compromised immune systems, shortened life spans and obesity. However, the incidence of these abnormalities appears dependent upon the species, particular genotypes and various nuclear transfer and embryo culture protocols used. While there may be some phenotypic differences detectable in the surviving clones, initial evidence suggests that these are not transmitted to progeny following sexual reproduction. This indicates that there are no obvious deleterious recessive genetic or epigenetic traits transmitted by clones. It also implies that any epigenetic differences in gene expression present in the clones are corrected during gametogenesis, providing some confidence in those applications that aim to capture the benefits of breeding from clones. In this regard, the cloning of elite ram or bull teams from progeny-tested sires for natural mating would be an effective means of disseminating genetic gain and hence productivity to farmers. Cloning can also be integrated into breeding strategies aimed at preserving endangered breeds of livestock and resurrecting valuable genetics identified following post-slaughter carcass evaluation. A major application of cloning is in conjunction with genetic modification. Specific genetic enhancements can be stably integrated into the genomes of cultured cells growing in the laboratory and nuclear transfer then used to generate cloned-transgenic livestock. The continual advances in animal genomics towards the identification of genes and their regulatory sequences that influence livestock production traits and human health will increase the ability to genetically modify animals to enhance efficiency and produce superior quality food and biomedical products for niche markets. The latter includes specialist dairy herds producing high-value human pharmaceutical proteins in their milk to treat specific diseases following purification and rigorous clinical testing; pigs whose tissues and organs are immunologically compatible with human patients, preventing hyperacute rejection following xenotransplantation; and the generation of animal models for particular human diseases to test new therapies. In addition, the technology is moving towards human therapeutic cloning and cellular trans-differentiation to provide cell- and gene-based therapies for regenerative medicine.

Genetic therapy approaches: laboratory tool and therapeutics

M Kalev. University of Auckland, Auckland

Gene transfer alters the instruction set of a cell through administration of pieces of DNA. When this approach is utilised for a therapeutic purpose, the term genetic therapy is used. Such manipulation causes the cell to acquire a new phenotype, determined by the introduced genetic material. Genetic therapy endeavours to apply advancing genetics knowledge to the treatment of human disease. It is a burgeoning field in which 987 clinical trials are currently registered.

All gene therapy conducted to date has been on specific populations of somatic cells, so that introduced genetic material can not be passed on across generations. This approach is strictly guarded by the Moratorium on germ-line gene therapy.

Genetic transfer was originally envisaged to provide a cure for diseases identified as resulting from a single gene defect (e.g. haemophilia), where delivery of a functional copy of the gene could provide a therapeutic benefit. However, it is now apparent that this approach can be utilised in diseases which do not have a single or well-defined genetic component. Most current genetic therapy trials target acquired diseases, in particular cancer. The emerging application is for silencing of abnormal genetic aberrations - inherited and acquired.

Success of gene therapy is dependent on efficient and specific gene transfer into the target cell. Both viral and non-viral delivery techniques are under development. Our laboratory uses an adeno-associated viral vector to deliver genes into cells in the brain. We showed that resetting neuronal circuitry by delivering the glutamic acid decarboxylase enzyme into the subthalamic nucleus, improves symptoms of Parkinson Disease. This approach has been taken by the substantial combined efforts of the members of our laboratory from the concept, to cells, to animals and finally to the phase I genetic therapy clinical trial. Six patients with severe Parkinson Disease have now undergone the procedure at the New York Weill Cornell Medical Center.

Lymphoproliferative diseases - the role of morphological and immunophenotypic analysis

B Bain. St Mary's Hospital, London, UK

The term 'lymphoproliferative disorder' (LPD) is conventionally applied to chronic lymphoid neoplasms, either leukaemia or lymphoma, of B, T or NK lineage. Cytology and immunophenotyping are of crucial importance in the diagnosis of the chronic lymphoid leukaemias and of non-Hodgkin's lymphoma (NHL) presenting in leukaemic phase. As B-lineage chronic lymphocytic leukaemia (CLL) is the LPD most often involving the blood, it is useful to initially apply tests appropriate for making a distinction between CLL and NHL, followed by any further tests that then appear indicated. A useful initial immunophenotyping panel, recommended by the British Committee for Standards in Haematology is CD2, CD19, CD5, CD23, CD79b, anti-kappa and anti-lambda (Table 1).

Table 1. Panel of antibodies for the diagnosis of mature lymphoproliferative disorders

B-lymphoid	T-lymphoid	B and T lymphoid	Oncoprotein-associated
First line CD19, CD23, FMC7, SmIg (anti-kappa and anti-lambda), CD22, CD79b	CD2	CD5	
Second line CD11c, CD103, HC2*, CyIg, CD79a, CD138	CD3, CD7, CD4 CD8	CD25	Anti-cyclin D1

Optional markers: CD11b, CD16, CD56, CD57, TdT, TIA-1

*CD123 could replace HC2 since HC2 is not commercially available

EMERGENCY PANEL

B-lineage lymphoproliferative disorders

B-lineage LPD show expression of surface membrane immunoglobulin. There is light-chain restriction, i.e. there is expression of kappa or lambda light chain but not both.

Chronic lymphocytic leukaemia

CLL has typical cytological features and a distinctive immunophenotype-SmIg weak, light-chain restricted, CD5+, CD23+. FMC7-. CD22/CD79b weak or -. The cytology and immunophenotype together are sufficient to confirm the diagnosis.

Prolymphocytic leukaemia (PLL)

PLL is usually readily diagnosed from the cytology and the immunophenotype. The neoplastic cells are larger than those of CLL and have a nucleolus, which-at least in the larger cells-is large and vesicular. The immunophenotype resembles that of NHL rather than that of CLL.

Hairy cell leukaemia

Hairy cell leukaemia (HCL) is usually readily diagnosed on the basis of cytology, bone marrow histology and the specific immunophenotype. Hairy cells are positive for CD11b, CD25, CD103 and HC2 (or CD123). The tartrate-resistant acid phosphatase reaction is still useful for laboratories without easy access to this panel of monoclonal antibodies.

Follicular lymphoma

It is not uncommon for patients with follicular lymphoma (FL) to have circulating lymphoma cells, even at presentation. The lymphoma cells are often smaller than those of CLL and they may have very scanty cytoplasm. Chromatin is more evenly condensed. Some of the cells have nuclear notches or clefts, the latter being deep with parallel edges. Smear cells are infrequent. The typical immunophenotype is SmIg strong, CD5-, CD23-, FMC7+, CD22+ and CD79b+. CD10, if positive, is diagnostically useful since it is not so often positive in other chronic LPD.

Mantle cell lymphoma

Mantle cell lymphoma is readily distinguished from typical CLL on the basis of cytology but may be confused with CLL, mixed cell type. It is a difficult morphological diagnosis because there are no really distinctive identifying features. Cells tend to be pleomorphic and some have quite an immature chromatin pattern. The typical immunophenotype is very different from that of CLL and closer to that of other NHL. Typically it is SmIg strong, CD5+, CD23-, FMC7+, CD22+, CD79b+ and CD10-. Cells express cyclin D1, which can be detected by immunohistochemistry or by flow cytometry (with a permeabilisation technique). Expression of CD5 and cyclin D1 are the most useful features for making a distinction from other NHL.

Splenic marginal zone lymphoma including splenic lymphoma with villous lymphocytes

Splenic marginal zone lymphoma (SMZL)-including splenic lymphoma with villous lymphocytes (SLVL)-can be confused cytologically with CLL. The cells are small and mature with scanty cytoplasm. The cytoplasmic margins are often but not always more irregular than is typical in CLL. These 'villous' projections may be polar, but this is not always so. There may be inconspicuous nucleoli. There are usually some cells showing plasmacytoid features. The immunophenotype is that of NHL. There is usually no expression of CD5 or cyclin D1. SLVL may express CD11c and in a minority of cases there is also expression of CD38.

Lymphoplasmacytic lymphoma

In lymphoplasmacytic lymphoma there are some cells with plasmacytoid features, more plentiful cytoplasm than in a small lymphocyte, eccentric nucleus and Golgi zone. There may also be features attributable to a paraprotein such as rouleaux formation or cold agglutination. The immunophenotype is typical of NHL with no expression of CD5 or cyclin D1. Some neoplastic cells may have immunophenotypic features of plasmacytoid differentiation such as cytoplasmic immunoglobulin (clg), CD38 or CD138.

T-lineage and NK-lineage lymphoproliferative disorders

T-lineage LPD usually show expression of CD3 and there are rearrangements of T-cell receptor (TCR) genes. The immunophenotype may be suggestive of clonality but is less definitive. Other T-cell markers expressed often include CD2, CD4 and CD5. In T-prolymphocytic leukaemia (T-PLL) there is usually CD7 expression and in adult T-cell leukaemia/lymphoma (ATLL) CD25 is usually expressed. T-lineage large granular lymphocyte leukaemia (T-LGL) differs in that there is usually expression of CD8 but not CD4 and markers of cytotoxic T cells, such as CD16 and CD57 and sometimes CD11b.

Normal NK cells and NK-LGL cells do not express CD3 and do not rearrange TCR genes.NK LGL usually expresses CD2, CD16 and CD56. There is variable expression of CD8, CD11b and CD57.

Conclusion

By a judicious combination of cytology with other techniques it is often possible to make a precise diagnosis of a lymphoproliferative disorder from the peripheral blood and bone marrow. Cytogenetic or molecular genetic analysis can be confirmatory. Lymph node or other histology is not always necessary.

Lymphoproliferative case studies

G Corbett. Waikato Hospital, Hamilton

Chronic lymphocytic leukaemia (CLL) is a B-cell clonal lymphoproliferative disorder characterised by the presence of a lymphocytosis in the peripheral blood, typically with small lymphocytes and some smeared cells. The diagnosis is confirmed by flow cytometry. The disease is staged at presentation on the basis of clinical and blood findings. More recently the Binet classification system has been used in most clinical trials. This separates patients into 3 groups on the basis of cytopenias and lymph node assessment. Stage A disease is characterised by the presence of blood and marrow lymphocytosis and less than three areas of palpable lymphoid tissue enlargement, and carries a median survival of > 7 years. In stage B disease there are three or more areas of palpable lymphoid tissue enlargement and it carries a survival of < 5 years. In Stage C disease there is anaemia (Hb <110g/l in men and < 100g/l in women) or platelets < $100x10^9$/L. Stage C disease carries a prognosis of < 2 years. At present it is recommended that treatment be reserved for patients with progressive Stage B and with Stage C disease. These recommendations may change as more is becoming known of the biology of the disease and novel and potentially curative treatments become available. Recently, other ways of assessing prognosis in CLL have become available. This includes measuring cytogenetics on the peripheral blood or marrow. This can be achieved relatively simply for the most common known cytogenetic abnormalities using Fluorescence in situ hybridisation (FISH) on the peripheral blood. Cytogenetic abnormalities may be found in approximately 80% of CLL cases. About 15% of cases express trisomy 12. Other abnormalities with poor survival are 11q and 17 p deletions. About 35% of patients have a deletion of chromosome 13, which carries a good prognosis. The expression of CD38 (>20% of cells) on CLL cells has been shown to be a poor prognostic feature. More recently it has also been shown that a lack of Heavy chain variable gene mutation in CLL cells carries a poor prognosis. This appears to be mirrored by the expression of the protein tyrosine kinase, ZAP-70. With the availability of some new treatments for the management of CLL it is desirable to monitor the patient's progress by clinical assessment and by peripheral blood findings. It is also helpful to be able to monitor minimal residual disease if autologous stem cell

collection is to be considered. In that situation it is desirable to clear the bone marrow of as much tumour as possible. Beyond evaluation of bone marrow and trephine biopsies morphologically it is possible to monitor residual disease using Immunoglobulin gene rearrangement studies on the peripheral blood and also using flow cytometry. The recent availability of 4 colour flow cytometry makes this more feasible. In the course of time patients may die from infection or bone marrow failure. About 10% of patients' progress to high grade Non Hodgkins lymphoma (Richter's transformation) Transformation to PLL may occur in approximately 10%. Rare cases of transformation to ALL and Hodgkins disease have been reported.

The glycolipid chemical basis of subgroups of blood group A

S Henry. Auckland University of Technology, Auckland

Purpose

Although quantitative and qualitative arguments have been debated the chemical basis for the weak ABO subgroups has been largely unexplored.

Methods

Glycolipids were isolated from individuals of a range of rare A-weak subgroups. Blood groups were determined serologically and confirmed by ABO, Lewis and Secretor genotyping and ABO sequencing. Immunochemical thin-layer chromatography staining techniques using panels of mapped monoclonal reagents were used to stain chromatographically separated blood group A structures.

Results and conclusions

Semi-characterised monoclonal antibodies revealed that in addition to the expected quantitative differences between common phenotypes and the weak subgroups, qualitative glycolipid differences, or at least an apparent qualitative basis due to major changes in ratios of different structures. Specifically it was found that the weakest A expressing samples (Ael phenotype) appeared to express an unusual A structure in the 8-12 sugar region. Variable expression of several structures in one of the A-weak samples and an AfinnB sample were suggestive of novel blood group A structures. Although no structural characterisation could be undertaken, the results are clearly indicative that the variant glycoslytransferases of the rare ABO subgroups, are not only inefficient but they may potentially synthesise novel ABO structures.

Synthetic ABO molecules inserted into group O cells for use as ABO quality control cells

S Henry. Auckland University of Technology, Auckland

Background

Until recently there have been no standardised control cells for ABO blood grouping. Recent innovations (described in international patent applications) have resulted in processes which allow cells to be created which express controlled levels of ABO blood group antigens.

Methods

Synthetic A and B KODETM molecules were used to transform O red cells to produce red cells expressing low levels of blood group A and B antigens. These transformed cells were formulated to simulate a routine patient sample in appearance and performance. A clinical scenario was developed that would allow a KODETM weak B and three different strengths of KODETM weak A cells to be presented for testing.

Results and conclusions

These samples were utilised as an educational exercise by the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Educational Exercise Survey that is tested by 310 laboratories in the Asia-Pacific region. Laboratories were asked to perform routine blood group testing on the samples. The objectives of this field trial were to determine the suitability of the KODETM product in the field, determine the variation between laboratories and variation in analytical sensitivity between methods. Results were obtained from a wide range of reagents in tile, tube, column agglutination and on a range of automated instruments. A large range of laboratory performance issues were identified which highlighted the requirements for ABO sensitivity controls.

New concepts in microbiology

S Jaksic. Pathology Associates

Prospects for immunotherapeutic control of biofilm formation

A major component of most bacterial biofilms are extracellular polysaccharides which serve multiple functions in virulence and also contribute to resistance to host phagocytic defences. In the case of *Pseudomonas aeruginosa*, production of the extracellular polysaccharide alginate is critical to pathogenesis of chronic lung infections in cystic fibrosis (CF) patients. Opsonic antibodies to alginate provide protection for animals against *P.aeruginosa* infection and their presence in CF patients is associated in decreased respiratory tract infections. Natural exposure to alginate is a poor inducer of opsonic antibodies in humans, so a conjugate vaccine has recently been developed and evaluated - in mice high levels of opsonic antibody associated with protection from *P.aeruginosa* lung infection have been generated. In addition, fully human monoclonal antibodies to alginate have been produced; they provide protection against murine infection when given prophylactically. Immunotherapeutic interventions may provide an alternative to antibiotics for therapy of serious pseudomonal infections.

Phage lytic enzymes for the treatment of bacterial infections

Bacteriophage lytic enzymes are used by the phage to quickly destroy the bacterial cell wall to allow the release of bacteriophage progeny. Their rapid and lethal action has been investigated as a potential 'weapon' against pathogenic bacteria. Phage enzymes are specific for the species or strain from which they were produced, so their use would not affect the surrounding commensal organisms found on mucosal surfaces. Enzymes specific for *Streptococcus pyogenes, Streptococcus pneumoniae, Bacillus anthracis, Staphylococcus aureus, Enterococcus faecalis and E.faecium* and *group B streptococci* have been isolated and tested. Using any of the phage lytic enzymes in vitro, 107 bacteria can be killed within seconds. In a S.penumoniae septicaemia model, numbers of bacteria in the blood of infected animals are reduced by >3-logs with a single IV dose of enzyme. Phage lytic enzymes are new agents that may be used to safely and efficiently control pathogenic bacteria in blood and on mucosal surfaces.

Laboratory approach to H. pylori testing

C Mansell. Waikato District Health Board, Hamilton

Helicobacter pylori causes duodenal and gastric ulcers and some cases of gastric carcinoma and MALT lymphoma. Diagnosis, treatment and confirmation of cure are beneficial in these conditions. However, *H. Pylori* infection is common and most carriers are asymptomatic. Even among those with dyspepsia, only a minority seek medical attention. The role of *H. pylori* in non ulcer dyspepsia is uncertain and its eradication usually does not improve symptoms.

The gold standard for *H. pylori* diagnosis is histology and culture of biopsies taken by gastroscopy. Serological tests are imperfect but are satisfactory for initial diagnosis before treatment, if used within a planned management strategy. The urea breath test is well validated for pre-treatment diagnosis and for post-treatment test of cure. However, it has been difficult to confirm equivalent performance in clinical use because numbers of untreated patients and treatment failures available for enrolment have declined in recent years.

Guidelines for investigation of dyspepsia were developed for the Waikato region, taking into account the local prevalence of *H. pylori*, availability of gastroscopy and clinical gastroenterology services. General practitioners, gastroenterologists and referring laboratories were involved in the process and stool antigen testing has replaced the urea breath test in our practice.

Uncertainty of measurement

T Barker. LabPlus, Auckland Hospital, Auckland

The uncertainty of measurement (U of M) is defined as a parameter associated with the result of a measurement that characterises the dispersion of the values that could be reasonably attributed to the measurement. This parameter is a useful aid for the clinical interpretation of patient results. It is also a requirement of ISO 15189 that the laboratory shall determine and document the U of M where relevant and possible. The U of M is made up of many components, but those that are not already included in the coefficient of variation for the method are often either insignificant or cannot be easily measured. In contrast to the use of U of M for the manufacture of reagent, standard and control material, a highly accurate calculation of this parameter is neither possible nor required for medical laboratory test results, especially when its clinical use is considered. It should remain a simple calculation that indicates the overall reliability of an assay over a period of time, and one that can be easily reviewed if changes are made to the analytical method. The U of M also helps indicate the number of significant figures that should be used for reporting a result and for the reference interval that is quoted.

Microarray analysis in lymphoid malignancies

I Morison. Southern Community Laboratories, Dunedin

Every cell in the body contains a full compliment of approximately thirty five thousand genes, but the level of activity (expression) of these genes varies considerably from tissue to tissue and from cancer to cancer. When genes are active they produce RNA and, by using microarray technology, the level of RNA for every gene can be determined simultaneously in a single experiment. For each tissue, an expression profile which measures the relative activity of every known gene can be obtained. Comparison of the gene expression profiles of various malignancies can be used to sub-classify disease, discover new disease groups, identify diagnostic, prognostic and therapeutic markers, and identify possible drug targets. Significant contributions in the field of lymphoid malignancy include the identification of subsets of diffuse large cell lymphoma, identification of distinct biological sub-groups of childhood ALL and the development of prognostic markers in CLL.

Cellular therapy for lymphoid malignancies

D Ritchie. Malaghan Institute of Medical Research, Wellington

Lymphoma and lymphoid leukaemia continue as therapeutic challenges for haematologists and oncologists. Recent developments in the understanding of the biology of these diseases have resulted in a greater appreciation of the interplay between malignant lymphoid cells and the host immune system. This has lead to the potential application of humoral and cellular immunotherapies as either adjuvant or salvage treatment. Importantly, we now understand that the differentiation and maturation status of B cell lymphomas may result in either enhanced recognition by cytotoxic T lymphocytes (CTL) or alternatively suppression of CTL function. The implication of these phenotypic differences is that certain subgroups of lymphomas may be selected as prime targets for cellular immunotherapies whilst in others the treatment focus should remain on enhancing chemotherapeutic options.

The Ziehl-Neelsen stain: perils and pitfalls

R Lumb. Institute of Medical and Veterinary Science, Adelaide, Australia

For many countries, laboratory diagnosis of tuberculosis (TB) is essentially limited to the detection of acid-fast bacilli in sputum smears stained by the Ziehl-Neelsen (ZN) method. Even in countries such as Australia and New Zealand where budgets allow for 'state of the art' technologies, a properly performed ZN stain will allow smear-positive patients to be rapidly identified. Unfortunately, there are perils and pitfalls that may result in false-negative or false-positive results. A false-negative result may result in smear-positive patients not being identified, causing on-going disease and potential for transmission to the general community. False-positive results may result in treatment of persons who do not have TB, and on-going treatment for patients who have responded well to treatment. In some situations, a true-smear positive patient remains undiagnosed. There are numerous opportunities for errors to occur.

The rational use of nucleic acid amplification techniques for the laboratory diagnosis of mycobacterium tuberculosis complex direct from clinical specimens

R Lumb. Institute of Medical and Veterinary Science, Adelaide, Australia

Traditional laboratory diagnosis of tuberculosis (TB) suffers from the paradox of being rapid but insensitive (microscopy) or sensitive but slow (culture). For clinicians and TB Services, prompt recognition of patients with TB allows initiation of appropriate therapy and contact tracing protocols. Initially, the introduction of Nucleic Acid Amplification Techniques (NAAT) for the rapid detection of Mycobacterium tuberculosis complex (MTBC) promised a revolution in the timely identification of patients with TB, but reality has fallen short of the initial promise. It is now clear that a combination of clinical suspicion and culture is the most sensitive determinant for the diagnosis of TB. In contrast, NAAT appears to be establishing a niche role when a rapid diagnosis of TB, or otherwise, can influence decisions regarding treatment, recommendations for further invasive investigations to secure a diagnosis, and prompt initiation of contact tracing protocols. To provide a successful NAAT service, laboratories have to commit to appropriate infrastructure, trained staff, sufficient budget, and a consistent approach to accepting or rejecting specimens for testing. The current protocol for NAAT is based on the level of clinical suspicion, smear result, intention to proceed to more invasive investigations, and

modifications to patient management. The protocol was established after consultation with TB Services, Thoracic Departments and senior staff of the Infectious Diseases Laboratory.

Real-Time molecular diagnosis of human metapneumovirus

I Mackay, S Bialasiewicz, M Nissen, T Sloots. SASVRC, CMVC, Dept. of Infectious Disease, and Division of Microbiology,Royal Children's Hospital and University of Queensland, Brisbane, Australia

In June 2001, a new addition to the family Paramyxoviridae, human metapneumovirus (hMPV), was described by a group from the Netherlands. Despite the novelty of its description, serological data indicated that the virus had been circulating amongst ill humans for at least 46 years. Using gene sequences present on GenBank at that time, we developed primers for a conventional diagnostic RT-PCR targeting the hMPV nucleoprotein gene. The assay was used to screen our local hospital population for the presence of this emerging respiratory pathogen which was quickly found. Primary isolates of local hMPV strains grew slowly and caused limited cytopathic changes in culture thus molecular diagnosis was the best approach to detecting infection. Using a rapid and sensitive real-time adaptation of our RT-PCR assay we identified hMPV in 6%, 4% and 3.4% of the study population during 2001, 2002 and 2003 respectively. Upon sequencing portions of the genome from local hMPV strains we discovered a preponderance of one genetic lineage, hMPV type A. Subsequent comparison of our oligonucleotides to those from studies worldwide demonstrated that the majority of primers in use bias the detection of hMPV genetic lineages towards the type A strains. We have now developed new molecular assays that detect all known strains of hMPV by targeting conserved genetic regions of the phosphoprotein and matrix genes obtained from our own studies as well as by comparison to sequences residing on GenBank. These assays were designed to be performed in conventional or real-time PCR formats producing an amplicon that is ideal for genotyping hMPV strains. The new assays have greatly improved our ability to diagnose hMPV related illness resulting in increased detection rates and providing a simplified tool for studying the global distribution of hMPV infection.

Real time PCR detection of Herpes viruses

V Wells. Canterbury Health Laboratories, Christchurch

Purpose

Real time polymerase chain reaction (PCR), along with melting curve analysis, are rapid procedures for the detection and genotyping of herpes viruses in a routine diagnostic setting. We compared the performance of a real time PCR assay for herpes simplex virus (HSV) to virus culture and enzyme immunoassay (EIA), and for varicella zoster virus (VZV) in comparison with culture and immunofluorescence (IF).

Methods

A total of 156 mucocutaneous swabs in viral transport media were tested for the presence of HSV by real time PCR using the LightCycler, culture and EIA. A further 124 specimens were tested for the presence of VZV by PCR using the LightCycler, culture and IF.

Results

Overall, 55 specimens were positive by HSV PCR. In comparison, 46 specimens were positive by cell culture, and 27 by EIA. All 55 positive specimens were considered true positives, as they were positive by two or more tests. The sensitivity of the PCR compared to cell culture was 100% and a specificity of 92%, while the EIA had a sensitivity of 70% and specificity of 99%. A total of 43 specimens were positive by VZ PCR, 23 specimens were VZV culture positive and 32 positive by IF. Off the 43 PCR positive specimens, 11 were neither positive by cell culture or IF. Compared to cell culture the PCR had a sensitivity of 100% and specificity of 83% while the IF method had sensitivity of 92% and specificity of 91%.

Conclusions

The real time assays for HSV and VZ had an improved sensitivity over culture and IF or EIA. Additional advantages include a rapid processing time with the capability of typing for HSV.

Evaluation of a Lightcycler confirmatory assay for Chlamydia trachomatis

L Bennett. Medical Laboratory Wellington

Purpose

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI) in New Zealand, with up to 80% of cases being asymptomatic. The sequelae of untreated chlamydial infections can be severe. It is recommended that in lower prevalence populations, positive NAAT results be confirmed with an independent test. We describe a modified extraction protocol for Roche Amplicor CT/NG specimens and validation of a real-time PCR assay developed to confirm positive Chlamydia results.

Methods

Our protocol involved proteinase K digestion of the Amplicor DNA extract and subsequent High Pure column purification (Roche). Real-time PCR was carried out using hybridization probes and primers targeted to the Major Outer Membrane Protein (MOMP) (TIB MolBiol).

Results

Results of a study on 200 specimens shows that confirming positive Amplicor results with the MOMP assay (sensitivity 95.7%, specificity 100%, PPV 100%, NPV 96.4%) improves the agreement between results (98% vs 94%) when compared to our current algorithm, which confirms with a repeat Amplicor test (sensitivity 88%, specificity 100%, PPV 100%, NPV 89.3%).

Conclusions

Confirming results with the MOMP assay is more robust than with Amplicor alone, and reduces reporting time. Additionally, the MOMP assay produces a lesser number of clinically unhelpful equivocal results.

Myb oncogene regulation of diverse target genes

G Lang. Institute of Cancer Research, London, UK

Hematopoiesis, the process by which mature blood cells arise, is controlled by multiple transcription factors, which act in stage and lineage-specific complexes. It is a major goal to elucidate the genes regulated by these transcription factors, in order to obtain a full understanding of the process and its malignant counterpart, leukaemia. Myb family transcription factors play a central role in hematopoiesis. To identify new Myb target genes we have applied an inducible dominant negative protein to subtraction cloning, in a model cell system, FDCP-Mix, with many characteristics of normal hematopoiesis. We identified a novel group of 30 validated Myb target genes of diverse function.

Eosinophilia: a case study

H Pullon. Waikato Hospital, Hamilton

Primary Hypereosinophilic Syndrome (HES) or Chronic Eosinophilic Leukaemia (CEL) is a rare myeloproliferative disorder of unknown aetiology. It can however present insidiously, with a number of serious and life-threatening complications.

The case of a New Zealand Caucasian man who developed HES is presented. His initial diagnosis was delayed because he worked as a freezing worker and at first his high eosinophil count was attributed to exposure to animal parasites. He was subsequently found to have marked eosinophilic proliferation within the bone marrow, moderate splenomegaly, and significant endomyocardial fibrosis with tricuspid regurgitation and right heart failure. He was initially treated with Hydroxyurea, before being changed on to low dose subcutaneous alpha interferon. He subsequently underwent major cardiac surgery which involved endomyocardial stripping and tricuspid valve replacement.

Although alpha interferon achieved good control of his eosinophil count, he subsequently developed significant interferon-related side effects including asthenia and muscle wasting, depression, and chronic liver dysfunction. His cardiac status has stabilised, although he continues to have some degree of tricuspid incompetence, mitral regurgitation, and mild right heart failure with chronic hepatic congestion. He remains on Warfarin long-term.

Treatment with low dose oral Imantinib (Glivec) has been shown to be very effective in many patients with primary HES, although unfortunately is not yet funded for patients with this condition in NZ. Nonetheless Exceptional Circumstances funding for such treatment is being sought for this patient.

Eosinophilic leukaemia and the idiopathic hypereosinophilic syndrome

B Bain. St Mary's Hospital, London, UK

Hypereosinophilia may be identified as reactive or neoplastic or the cause may be unknown. Reactive eosinophilia is eosinophilia that is cytokine driven and that occurs as a response to an underlying disease or allergen exposure. Identifiable causes include allergies such as hay fever, eczema and asthma, drug allergies and parasitic infections. Reactive eosinophilia may also be a response to an underlying neoplastic condition such as lymphoma (Hodgkin's disease or non-Hodgkin's lymphoma), acute lymphoblastic leukaemia, carcinoma or sarcoma. In other patients, eosinophilia is the result of a myeloid neoplasm and the eosinophils are part of the neoplastic clone. In Philadelphia (Ph)-positive chronic myeloid leukaemia, for example, the majority of patients have an absolute eosinophilia and these eosinophils carry the Ph chromosome. Similarly, in other chronic myeloproliferative disorders, such as idiopathic myelofibrosis, the eosinophils are clonal. There are also a few subtypes of acute myeloid leukaemia (AML) where differentiation characteristically includes eosinophils. This is so in AML associated with t(8;21) or inv(16). The term eosinophilic leukaemia is used for a chronic myeloproliferative disorder where eosinophilic differentiation predominates. The term idiopathic hypereosinophilic syndrome (idiopathic HES) is a diagnosis of exclusion. It refers to a condition in which eosinophilia remains unexplained despite the application of all modern diagnostic methods. By definition, the eosinophils cannot be shown to be clonal and no underlying disease that provides an explanation for the eosinophilia can be found. Recent advances in understanding hypereosinophilia In the last few years the proportion of patients with eosinophilia who are assigned the diagnosis 'idiopathic HES' has lessened. Some patients have been found to have a clone of abnormal cytokine-secreting T cells and the eosinophilia is therefore recognized as reactive. In another group, clonality has been demonstrated by the discovery that there is an interstitial deletion of chromosome 4 that leads to formation of a chimaeric oncogene (FIP1L1-PDGFRA) and eosinophilic leukaemia. This is a submicroscopic event, with conventional cytogenetic analysis being normal.

Diagnosis of eosinophilic leukaemia

Eosinophilic leukaemia can now be diagnosed when there is eosinophilia with evidence that the disorder is leukaemic in nature. Such evidence may be (i) an increase of blast cells in the blood or bone marrow (ii) the presence of a clonal cytogenetic abnormality or (iii) the presence of a clonal molecular genetic abnormality such as the FIP1L1-PDGFRA fusion gene. The presence of hepatosplenomegaly is suggestive but not conclusive Precise diagnosis is important since some eosinophilic leukaemias respond to Imatinib, the drug that is showing such promise in Ph-positive chronic myeloid leukaemia. This is so not only for cases with FIP1L1-PDGFRA fusion but also for those with rearrangement of the PDGFRB gene.

Avian influenza in Asia: are we ready for the inevitable?

L Jennings. Canterbury Health Laboratories, Christchurch

Emerging and re-emerging infectious diseases are an ever-present global concern. The most recent SARS coronavirus (SARS-CoV) infections in April 2004, in China, associated with a breach in laboratory protocols, have again shown that if quick action is taken, SARS is a containable disease. However, the epidemic of avian influenza caused by the highly pathogenic avian influenza A(H5N1) virus is still spreading, despite containment efforts, through domestic poultry and a variety of other birds in Asia. This epidemic is unprecedented, both for its immense geographical scale, affecting poultry in eight Asian countries since late 2003, and for its human health implications. The H5N1 2004 virus is antigenically and genetically distinct from the 1997 virus, which caused the 'Chicken Flu' outbreak in Hong Kong and is thought to have evolved from an A/Goose/Guangdong/96-like virus. In addition it is now highly pathogenic for a wide range of species. Initial seeding through Asia may have been by wild migratory bird species, however, the commercial and other movement of live poultry between Asian countries, along with the live bird and wet market practices may have provided the environment for amplification and the wider dissemination in this outbreak. Human cases with a high fatality rate have been reported in Viet Nam and Thailand. Although human-to-human transmission of this virus has not been confirmed, as long as A/H5N1 continues to circulate in the poultry population, the emergence of a new virus strain, with pandemic potential remains. The co-infection of A(H5N1) with influenza A viruses circulating in pigs or humans are possible routes for the evolution of such a novel virus. The control of this current avian epidemic is essential to prevent further human outbreaks and avert a human influenza pandemic. Lessons learnt following the 1997 Hong Kong outbreak show that rapid culling followed by the introduction of biosecurity measures including hygiene were pivotal to the control of that outbreak and subsequent virus circulation. New lessons from the current 2004 outbreak indicate that collaboration between animal and human health sectors is essential and needs to be developed in many countries. Early warning systems and sentinel surveillance are core activities, however the challenge is how to shorten the 'astute' clinician's observation of something

different going on, to identification of the problem by the public health sector and implementation of investigation and control measures.

Real time PCR quantification of Epstein-Barr virus DNA in transplant patients

R Nagappan, M C Croxson, A Wesley. Department of Virology, Lab Plus, and Department of Gastroenterology, Auckland Hospital, Auckland

Epstein-Barr virus is a member of the herpes family with a large genome of 170kb. Primary infection with this virus is usually subclinical. The most common symptomatic primary infection is acute infectious mononucleosis, a self-limited clinical syndrome. As with other herpes viruses EBV remains latent in cells. Latent EBV may reactivate and has been implicated in lymphoproliferative disease, Burkitt's lymphoma and Nasopharyngeal carcinoma. Infection with EBV poses a serious threat to immunosuppressed patients. Post transplant lymphoproliferative disorder (PTLD) encompasses a spectrum of EBV associated diseases that may progress to fulminant lymphoma if left untreated. Serial quantitation of EBV can be used as a predictive marker for the development of PTLD and as a measure of response to treatment. It is still uncertain what level of EBV DNA indicates uncontrolled EBV proliferation. In order to define "high" viral load, there is a need for laboratories to standardize measurement of EBV copy numbers and to correlate this with clinical disease. Using Real time PCR we have developed a rapid, sensitive, specific and reproducible method for the detection of Epstein-Barr virus DNA. The assay can detect from 3 to107copies of EBV DNA with a wide linear range. From July 2003 a total of 27 patients are being monitored for EBV DNA fluctuations in peripheral blood mononuclear cells (PBMNC) and plasma.

Use of real-time PCR for detection of enteric viruses in environmental samples

J Hewitt, GE Greening. Institute of Environmental Science & Research Ltd, Porirua

Real-time PCR and RT-PCR were used to determine the presence and persistence of human enteric viruses in environmental samples. Real-time assays using specifically designed 5'exonuclease probes were carried out in a Corbett RotorGene. Several research studies have been carried out including examining the effect of acid marination and heat on norovirus and hepatitis A virus in mussels. Viruses were recovered from the shellfish matrices, then analysed using real-time RT-PCR and results compared to cell culture where possible. Using standard curves generated from stock hepatitis A virus or from a faecal suspension of norovirus, the real-time assays were able demonstrate changes in virus numbers following the effects of acid marination and heat treatment on the different viruses. In other research studies, we determined the time required for norovirus to be naturally removed or depurated from oysters growing in normal environmental conditions. Real-time RT-PCR has also been used to determine the prevalence of adenoviruses and enteroviruses in New Zealand shellfish, as well the presence in raw and treated sewage samples.

Cord blood banking

M Birdsall, Cordbank, Auckland

Cord blood has been increasingly recognised as a valuable source of haemopoeitic stem cells. More than 3500 cord blood transplants have now been performed, including more than 500 adult cord blood transplants. Cord blood stem cells have been used to treat a wide variety of conditions including haematological malignancies, inborn errors of metabolism, thalassaemia, sickle cell anaemia and neuroblastoma.

There is emerging evidence that cord blood may also contain stem cells for other cell types such as brain, liver or heart cells. There is exciting research using cord blood stem cells in a regenerative role post injury.

Cordbank is New Zealand's first cord blood bank and provides Kiwi parents with the opportunity to bank their babies' cord blood stem cells. Cordbank opened 2 years ago and more than 1000 cord blood units have been banked. To date, no units have been transplanted.

Changing trends in autologous transplantation

H Pullon. Waikato Hospital, Hamilton

Following the initial pioneering of Allogeneic Bone Marrow transplantation (BMT), techniques for the successful cryopreservation and storage of bone marrow were developed in the early 1980's. This allowed the use of "dose-escalated" chemo- and radiotherapy, with the potential for myelo-ablative bone marrow toxicity being averted by the re-infusion of previously harvested bone marrow stem cells. Hence the concept of autologous bone marrow transplantation (ABMT) or autografting was born.

At first the use of ABMT was explored in patients with Acute Myeloid Leukaemia (AML), Hodgkin's and Non-Hodgkin's Lymphoma, diseases where AlloBMT had already proven successful. But it was not long before the concept of dose intensification and subsequent autograft was being looked at in a whole range of malignant and non-malignant diseases.

In the late 1980's, flow cytometery, and in particular the use of CD-34 to accurately and quickly quantify stem cell numbers, resulted in more satisfactory and predictable engraftment, and a reduction in procedure-related deaths. In addition the successful isolation and commercial production of the cytokines G- and GM-CSF opened the door to successfully mobilising bone marrow stem cells into the peripheral blood. By the early 1990's Peripheral Blood Stem Cell (PBSC) harvesting began to replace bone marrow harvesting procedures, particularly when it was found that the use of PBSCells resulted in more rapid platelet and neutrophil engraftment.

Nowadays, in the 21st century, autografting using PBSCells is an integral part of haemato-oncological practice, and is routinely used in treating patients with Hodgkin's and non-Hodgkin's lymphoma, Multiple Myeloma, AML, Germ Cell Tumours and a variety of auto-immune diseases. Interestingly its use in poor prognosis Breast Cancer and AML is declining, whilst its application in the Chronic Leukaemias and autoimmune diseases is being increasingly explored.

The pre-analytical phase - an important area for quality improvement in the laboratory

B K Smith. BD Diagnostics, Australia

The face of the clinical laboratory has changed in many ways over the past few decades. Key changes have included the introduction of ever more sophisticated instrument systems. Often, these utilise technologies that were previously restricted to the realm of the research laboratory. Concurrent with this hardware development (and associated computerisation of clinical laboratories) we have

seen significant advances in the overall quality of laboratory services. To a large extent, these advances have been the consequence of improved quality systems in the laboratory and the superior precision and accuracy performance offered by modern automated laboratory analysers. To some extent at least, quality improvement in the pre-analytical phase has taken a 'back seat' to the above improvements within the laboratory. This is somewhat paradoxical given that the increased sophistication of many modern instrument platforms demands higher quality specimens. With the pre-analytical phase accounting for more than 50% of labour costs and processing time in many laboratories, a case can be made to devote more attention to this component of clinical laboratory processes. With consistently high standards of analysis now a reality in the majority of clinical laboratories in the developed world, future significant gains in overall quality of laboratory services are likely to come from improvements in the pre-analytical phase.

Paediatric blood collection - how do we define success?

B Smith. BD Diagnostics, Australia

Merely obtaining the required blood volume, by whatever means, in the frequently difficult setting of a busy paediatric unit is often regarded as defining a successful procedure. The fact is that success should be defined in terms of the safety of the procedure (for both the patient and the healthcare worker) and the quality of the specimen obtained. Obtaining a high quality specimen that is truly representative of the in vivo status of the patient's blood at the time of collection can be difficult - particularly with regard to collection of capillary blood. Nevertheless, procurement of such specimens is essential if laboratories are to produce accurate and therefore clinically useful test results. Optimum patient care and patient outcomes are dependent on this. The degree to which pre-analytical error adversely affects the quality of capillary blood specimens (and therefore test result accuracy) is poorly understood by many healthcare workers. The degree to which pre-analytical error can impact negatively on capillary blood specimens is poorly understood by many healthcare workers.

From rare disease to vaccination - New Zealand's meningococcal story

D Martin. Kenepuru Science Centre, Porirua

For the last 13 years New Zealand has experienced an epidemic of meningococcal disease during which the pre-epidemic population rate of 1.5 per 100,000 in 1989-90 soared to a peak of 17.4 per 100,000 (650 cases) in 2001. In 2002-2003 case numbers averaged 549. Since 1990 over 5400 people have contracted the disease and 220 (4.1%) have died. One in five survivors have suffered serious disabilities, such as limb amputations, or neurologic damage. In stark contrast most industrialised countries of the world report fewer than 3 cases for every 100,000 people each year. In 1991, at the start of the epidemic ESR collected meningococci from cases for the monitoring of antimicrobial resistance. Notifiable disease data was poorly collected and numbers were counted only at the end of each year. The meningococcal disease epidemic brought about the integration of national laboratory and notifiable disease data, ensuring that notification of cases occurs and near 100% of meningococcal case isolates are referred. A policy of giving antibiotics to suspected cases of disease, prior to admission to hospital, was introduced in 1995. This policy undoubtedly saved lives but affected the recovery of meningococci from cases. However, the introduction of PCR technology enabled detection of meningococcal DNA from patient specimens. PCR was followed by the use of DNA sequence typing to enable recognition of important gene sequences used to type meningococci and to establish the clonal nature of the epidemic strain. Antigens detected by serotyping are those important in eliciting protective immune responses. The demonstrated stability of the major PorA antigen on the B:4:P1.7b,4 strain during the course of the epidemic assisted the decision to use a strain-specific vaccine epidemic control. Over the last two years a vaccine, manufactured by Chiron Vaccines in association with the Norwegian Institute of Public Health, has been trialled in New Zealand in varying age groups. The immunogenicity data from these trials suggest that the MeNZB TM is immunogenic and likely to confer immunity against systemic group B meningococcal disease. Licensure of the vaccine will enable delivery of the vaccine to the wider population under the age of twenty years.

Procalcitonin testing in possible meningococcal disease - does it aid decision making?

G Mills. Waikato Hospital, Hamilton

Procalcitonin is currently regarded as a relatively bacterio-specific marker that increases rapidly (>4 hours) after an endotoxin challenge and is associated with high sensitivity for generalised bacterial sepsis and meningitis. It is not established as a routine diagnostic test in most hospitals in New Zealand. The objective of our first study at Waikato Hospital was to prospectively investigate the diagnostic characteristics of procalcitonin as an aid in the diagnosis of meningococcal disease in febrile young adults presenting to the our emergency department during the current sustained meningococcal epidemic.

Methods

The study population were emergency department patients aged 14-40yrs presenting with either a temperature 38.0 C without an obvious focus of infection, or symptoms consistent with meningococcal disease. All had procalcitonin levels, *N. meningitidis* PCR, blood +/- CSF cultures.

Results

183 patients presented with undifferentiated adult febrile illness over a nine-month study period. Nine were subsequently shown to have meningococcal disease. A positive procalcitonin (>0.5ng/ml) had a sensitivity of 100% (CI 66.4-100), specificity 89% (CI 83.1 - 93.1), NPV 100% (CI 97.6 - 100) & PPV 32% (CI 15.9 - 52.4) for meningococcal disease.

Conclusions

The likelihood ratio for a procalcitonin level >0.5ng/mL in this setting is 9.1. As a result, the finding of a procalcitonin level >0.5ng/mL in young adults with undifferentiated fever indicates a significantly increased likelihood that the presenting illness is meningococcal disease compared to the pre-test probability. In New Zealand's continuing meningococcal epidemic empiric antibiotics should be strongly considered in those with elevated procalcitonin levels in the hope of reducing meningococcal disease deaths due to delays in antibiotic administration.

Reporting haematology results - redundancy in the full blood count

I Morrison. Southern Community Laboratories, Dunedin

The 'full blood screen' or 'complete blood count' has evolved to

include a wide variety of parameters that undermine the professionalism of the modern clinical laboratory. It often includes redundant and non-contributory information that should not be reported. Provided the results from a haematology analyser are critically reviewed by experienced staff, all necessary information can be conveyed using the haemoglobin, MCV, platelet, neutrophil, monocyte, eosinophil and lymphocyte counts. In particular the concept of the total white blood cell count is a historical vestige that has arisen from the methodology used to count and identify blood cells. It does not comprise a gestalt measure of anything and has no place on a modern blood count report. Given that Greek is not a language well understood by clinicians, blood film descriptions should be avoided whenever possible. By reducing the number of components in a blood count and enhancing the interpretive value of blood film comments, the professional status of haematology technologists will be enhanced.

Moh's surgery

S Meharry. Diagnostic Medlab, Auckland

Mohs micrographic surgery (Mohs surgery) is a precise tissue-sparing surgical technique used in the removal and treatment of selected malignant neoplasms of the skin. This surgery requires a single surgeon to act in two distinct roles: surgeon and pathologist. The procedure is done in stages with succesive stages being used to remove extensive tumours as needed. The majority of simple skin cancers can be managed by simple excision or destruction techniques. Mohs surgery is chosen because of the complexity (e.g. poorly defined clinical borders, possible deep invasion), size or location (e.g. maximum conservation of tumour-free tissue is important). Mohs surgery is usually an outpatient procedure done under local anaesthesia (with or without sedation).

A histological approach to dental research

S K McKenzie. Wellington School of Medicine and Health Sciences, Wellington

The Dental Research Group is involved in investigating various aspects of oral microbiology and dental health. Their research aims to study the microorganisms responsible for a range of oral diseases, to understand how the diseases are caused, and to devise strategies for prevention. For example, tooth decay (dental caries), is caused by the bacteria present in dental plaque. A Dental plaque is an oral 'biofilm'. These are complex bacterial communities of hundreds of microbial species, which coat the oral cavity, including the teeth. The Group use a laboratory model, which simulates the oral environment in vivo. The model is known as MAM "the multiple artificial mouth". This allows the physical, chemical, biological and molecular features of cultured biofilms to be monitored. Histology is one of a few techniques employed to study the structure of dental plaque biofilms. Alongside TEM and SEM, it is important for natural human plaque where biofilm structure/behaviour may be related to pathogenesis. It is also important in that effective oral therapies are likely to depend on biofilm architecture. This histology work has involved using an agarose/paraffin wax double embedding technique to enable the friable plaques, which can measure anywhere between 200 microns and 5 millimetres in thickness, to be processed routinely, allowing orientation and structure to be maintained. The use of stains such as Toluidine blue and Gram stains allow visualisation of the framework of the dental plaques. This in turn enables the Group to create a descriptive, quantitative model of oral biofilm structure.

A time for fungus

D H Ellis. Mycology Unit, Women's and Childrens Hospital, Adelaide, Australia

Today we are faced with a constantly changing pattern of fungal epidemiology; for instance there has been a marked decrease in the number of AIDS related mycoses in countries where HAART is available; there has been a noticeable increase in the number of non-albicans species of Candida causing candidaemia, although the selection of *C. glabrata* may now be declining and there have been increased numbers of mould infections caused by the likes of *Aspergillus, Scedosporium* and *Fusarium*. The increased incidence of these infections and the diversity of fungi causing them, has parallelled the use of more aggressive cancer and post-transplantation chemotherapy and the use of antibiotics, cytotoxins, immunosuppressives, corticosteroids and other macro disruptive procedures that result in lowered resistance of the host. Candida now accounts for 8-10% of all bloodstream infections with an annual incidence rate of 6-10 episodes per 100,000 population. Of those patients who develop nosocomial candidiasis, 35% will die from the infection and 30% will die from the underlying disease. Risk factors for the development of candidiasis and appropriate antifungal therapy often depend on the infecting species. Recent data from the Australian Candidemia study shows the following incidence figures for yeasts species isolated from blood; *C. albicans* (56%), *C. parapsilosis* (16%), *C. glabrata* (13%), *C. tropicalis* (5%) and *C. krusei* (5%). Antifungal susceptibility patterns for these yeasts were also predictable, importantly no isolates of the *C. albicans* isolates were considered resistant to fluconazole. Incidence figures for nosocomial mould infections are more difficult to obtain as these are non-reportable. Current estimates would be *Aspergillus fumigatus* (>70%), *A. flavus* (~4%), *Pseudallescheria boydii* (~4%), *Scedosporium prolificans* (~4%), *Fusarium/Acremonium* spp (~3%) and other moulds including the zygomycetes (~5%), although regional differences may be noted.

Determination of autoantibodies against granulocytes (ANCA) and glomerular basement membrane (GBM)

P Vogt, Euroimmun

The identification of autoantibodies against neutrophils (ANCA) is primarily based on indirect immunoflourescence and followed up by mono-specific ELISA and immunoblots. BIOCHIPs containing ethanol-fixed human granulocytes are used as a standard substrate for the immunofluorescence, allowing the differentiation of two ANCA types: the cytoplasmatic or 'classical' type (cANCA), which is associated with Wegener's granulomatosis and the perinuclear type (pANCA) which indicates a spectrum of various diseases, like microscopic polyangiitis, rapid progressive glomerulonephritis, inflammatory bowel diseases, primary sclerosing cholangitis, various vasculitis and other diseases. Main target antigens are proteinase 3 for cANCA and myeloperoxidase for pANCA. In some cases the differentiation between pANCA and antibodies against cell nuclei (ANA) in indirect immunofluorescence can be difficult. A BIOCHIP mosaic including BIOCHIPs coated with ethanol-fixed granulocytes, primate liver, formalin-fixed granulocytes and Hep-2 cells can be used as an highly advanced instrument for differentiation of ANCA and ANA. ANCAs can be detected in up to 30% of patients suffering from Goodpasture Syndrome. This syndrome is characterized by rapid progressive glomerulonenephritis and pulmonary haemorrhage, associated with anti-glomerular basement membrane antibodies

(GBM). These target a conformational epitope on collagen IV and are considered to be pathogenic. For the determination of GBM an immunofluorescence test with primate kidney and a monospecific ELISA should be performed in parallel.

ESR - Your mission should you choose to accept it...

H Poulsen, ESR Laboratories, Porirua

ESR's mission statement is 'Protecting People and their Environment through Science'. That just shows that the Forensic Group didn't fit in this CRI (Crown Research Institute) any better than any of the others that were formed in 1992. Forensic work means work for the Courts and it tends to deal with the people who weren't protected. In Forensic Toxicology we deal daily with the results of unexplained deaths, suicides, motor vehicle accidents, assaults and homicides. We use mainly GCMS (gas chromatography/ mass spectrometry) and LCMSMS (liquid chromatography/ mass spectrometry) to look for drugs or poisons that might have:

- caused death - prescribed and illegal drugs in intentional or accidental overdose
- been used to stupefy - date rape drugs or drink spiking
- altered mood - methamphetamine use in assaults and homicides affected the mind - antipsychotics and antidepressants, or lack thereof, in suicides
- alter psychomotor skills - alcohol, cannabis methamphetamine in traffic accidents

VTEC - New Zealand's experience

J M Bennett, C M Nicol, F M Thomson-Carter. ESR, Porirua

Purpose

Surveillance of VTEC/STEC in the community enables risk factors to be identified and outbreaks to be traced.

Methods

Diagnostic laboratories throughout New Zealand refer isolates of suspected VTEC/STEC to the Enteric Reference Laboratory (ERL) for confirmation, toxin-testing, and where appropriate, molecular typing. The EpiSurv electronic database is used by Population and Health Group ESR and community-based Health Protection Officers (HPO) to monitor trends. Statistics are supplied to the Ministry of Health monthly.

Results

There have been over 430 isolates of VTEC/STEC O157 and 28 non-O157 VTEC/STEC confirmed by ERL in the period 1993 (first isolate of VTEC O157) to 2003. No large common-source outbreaks have been detected but there have been small family clusters Retrospective molecular typing of all isolates since 2003 has shown clonal variation between the North and South Islands as well as within each island.

Conclusions

The rate of VTEC/STEC infection in New Zealand for 2003 was 2.8 cases per 100,000, which is higher than that reported by the USA (1.0) and England and Wales (1.3), but lower than the rate reported in Scotland (8.2). VTEC/STEC infection is a notifiable disease, which may have very serious sequelae. It is important that laboratories continue to screen for it and refer isolates for confirmation and possible epidemiological follow-up.

Bayesian approaches to diagnosis and receiver operating characteristic curve analysis

G Reeves, Hunter Area Pathology Service, Australia

The seamless incorporation of test results into the entire management process commences with correct question formulation & sensible test ordering and moves through the collection and analytical phases to timely, high-quality reporting and appropriate test interpretation and follow-up investigation. The Bayesian approach encompasses this ideal and encapsulates it mathematically.It involves the 'quantification of clinical acumen', and begins with an assessment of disease likelihood. Subsequent test results possess inherent characteristics based upon their sensitivity and specificity which dictate the likelihood of disease being present in the context of positive and negative results. Post-test likelihoods can be subjected, in an iterative fashion, to further follow-up testing to further clarify the possibility of disease. Receiver operating characteristic (ROC) analysis provides simple graphical and numerical correlates of overall test performance in this Bayesian setting. By portraying true positives graphed against false positives, the utility of a given test in clarifying disease probabilities starts to become apparent. The best ROC curve demonstrates a line hugging the upper & left hand side of the plot, with calculated area under the ROC curve (AUROC) of 1 being ideal, and with AUROC of 0.5 being no better than the toss of a coin. In certain circumstances, it may be desirable to 'exclude' or 'confirm' disease for screening and diagnostic purposes, respectively, In these settings, the positive likelihood of disease is best assessed at an optimal cut-off (Positive Likelihood at Optimal Sensitivity/Specificity, 'PLOSS'), represented by 100% sensitivity (for screening tests) and 100% specificity (for diagnostic purposes).

Coeliac disease and gluten sensitivity syndrome

G Reeves. Hunter Area Pathology Service, Australia

Coeliac disease (CD) is a fascinating hybrid condition, featuring aspects of dietary intolerance (to gluten found in wheat, barley, and rye) and autoimmunity (immune confusion with an attack on self-structures). CD has much to teach us about the genetic and environmental interactions that result in autoimmunity.

Classically, CD targets the small intestine, and presents with malabsorption characterised by weight loss, abdominal distension, diarrhoea and steatorrhoea. The median age of presentation is now 45, in contrast to the common misconception that this is still largely an infantile affliction.

The breadth of pathology induced by gluten can extend beyond the gastrointestinal tract, most notably causing the blistering itchy skin rash of dermatitis herpetiformis and the neurological complications of gluten ataxia. This range of clinical symptoms has prompted some authors to broaden the diagnosis from "gluten-sensitive enteropathy" to "gluten sensitivity syndrome".

The diagnostic "gold standard" requires demonstration of biopsy evidence of small bowel villous atrophy which normalises with dietary restriction and deteriorates upon rechallenge. This will allow most patients with symptomatic gut disease to be diagnosed. In most of the studies of coeliac serology to date, positive antibody results in the absence of demonstrable bowel pathology have been labeled falsely positive, thus reducing the diagnostic specificity of serology. It is likely that many of these patients with "normal" small bowel biopsies, but high levels of endomysial or transglutaminase antibodies, could be included in the category of gluten sensitivity syndrome if other relevant clinical features are evident (e.g. unexplained iron deficiency or

transaminitis). Particularly if these occur in the setting of an appropriate family history, IgA deficiency, or other immune conditions such as Type I diabetes or autoimmune thyroiditis.

A strict gluten-free diet not only improves symptoms and enhances nutrition and bone density, but it also may reduce the risk of developing other autoimmune conditions such as Type I diabetes, thyroid disease, and lupus. A Multi-Centre Coeliac Study, coordinated through Hunter Health and involving the expertise of seven major teaching centres throughout Australasia, is currently exploring optimal screening strategies while assessing variations in gut biopsy interpretations between pathologists. Assessment for a particular subtype of HLA-DQ (DQ2/DQ8), a gene involved in autoimmune conditions which has been found to be present in virtually all individuals with coeliac and diabetes, may soon allow refinement of screening for CD in selected individuals.

The rising incidence of autoimmune conditions parallels the increase in allergic problems, and has coincided with a reduction in infectious diseases, particularly in the developed world. These facts have led to the development of the "hygiene hypothesis", a theory suggesting that our improved hygiene deprives predisposed individuals of critical immune stimuli required to develop mature, well-adapted immune responses. Evidence in support of this theory is growing, but currently the data are circumstantial.

Exciting diagnostic and therapeutic changes await us in the management of CD. These include a potential role for genetic testing in clarifying the likelyhood and presence of CD, chemical treatments of gliadin to reduce its immune-activating potential and toxicity, and therapies designed to suppress the aberrant immune response. It is hoped that the Multi-Centre Coeliac Study will offer clarification of optimal strategies for serological screening within the coming 18 months.

Gut instinked

J Brooker, Waikato Hospital, Hamilton

Coeliac diaease (CD) is a common disorder affecting approximately 1% of the population in NZ. Immune mediated damage to the small intestine is stimulated by a common food antigen gluten found in wheat barley and rye cereals. This results in malabsorption of nutrients from the damaged small bowel particularly iron, folate and calcium. Characteristic patterns of antibodies in the blood are recognised as markers of CD and are useful in both diagnosis and to screen populations. Recognised histological lesions include atrophy of the villi, hypertropy of glands, increased inflammatory cells in the lamina propria and increased numbers of intra-epithelial lymphocytes. Prompt clinical improvement is noted with withdrawal of the responsible food antigens wheat barley and rye. However, some patients may have no symptoms or signs of CD and are only picked up on screening. Mechanisms of the immune mediated damage rely on the patients class 2 antigens HLA DQ2 interacting with gliadin a fraction of gluten. This leads to the formation of cytokines and antibodies. The cytokines damage the epithelial cells which leads to and increase expression of HLA DQ2 genes causing increased absorption of gliadin. The clinical features are malabsorption (diarrhoea and weight loss) anaemia abdominal pain, constipation osteopenia and dematitis herpetiformis (10% of CD). Diagnosis is suggested by antibodies to gliadin, endomysial antibodies and tissue transglutaminase. Endoscopic features include scalloping of the duodenal folds and flattening of the villi. Confirmation of CD is made on histology. In our recent retrospective study of 100 patients coming for upper GI endoscopy who had small bowel biopsies, 15 patients had CD and only 1 had negative serology and this patient was not IgA deficient.

Luminex(r) technology overview and autoimmune testing

E De Gooyer. Diagnostic Solutions, Australia

The Luminex(r) 100 is an exciting new system that presents laboratories with a new approach to diagnostic testing using the multiplexing xMAP(r) technology. The Luminex(r) will allow laboratories to assay for multiple tests from the one sample, in a rapid automated process. Luminex(r) sells the xMAP(r) technology through arrangements with exclusive partners covering a wide range of industries and specialties. These partners utilise the Luminex(r) 100 system, in conjunction with the Luminex(r) micro-beads, to provide kits for market specific applications. These applications include research / drug discovery and clinical diagnostics. Autoimmune antibody testing is an ideal clinical diagnostic application as frequently groups of tests are co-requested by clinicians in order to provide a more complete diagnostic analysis. Biomedical Diagnostics (BMD) is a French company, in partnership with Luminex(r), providing an autoimmune diagnostic application through their FIDIS(tm) System. The FIDIS(tm) System was launched in Europe in November 2003, and now has more than 100 systems installed around the world. The FIDIS(tm) assay panels available now include the Connective panel (9 tests), Celiac panel (3 tests), Thyroid panel (2 tests), and Rheuma panel (2 tests), with further autoimmune panels under development.

Immunophenotyping of haematological malignancies

S Ramachandran. LabPlus, Auckland Hospital, Auckland

Immunophenotypic analysis has contributed a great deal to the diagnosis of various haematological malignancies such as acute leukaemia, chronic lymphoproliferative disorders and lymphomas, and paroxysmal nocturnal haemoglobinuria (PNH). Morphological assessment, together with clinical information, provides a guide in selecting a suitable panel of antibodies to identify neoplastic cell populations in a variety of specimen types. Commercial monoclonal antibodies are used to detect surface, cytoplasmic and nuclear antigens or 'markers', on or in a cell. Acute leukaemia is a clonal disorder featuring maturation arrest with an accumulation of blast cells. A primary panel of antibodies permits the diagnosis of the majority of cases of acute leukaemias and their classification into the several major subtypes: acute myeloid leukaemia (AML), B and T-lineage acute lymphoblastic leukaemia (ALL), bi-phenotypic acute leukaemia, and undifferentiated acute leukaemia. The chronic lymphoproliferative disorders and lymphomas feature a clonal proliferation of lymphoid cells derived from one abnormal neoplastic cell. A panel of antibodies is used to discriminate a clonal vs reactive lymphoid population, and to designate the neoplastic cells into their respective B, T and natural killer cell (NK) lineages. Paroxysmal nocturnal haemoglobinuria is a clonal proliferation of somatically mutated haemopoietic stem cells, resulting in decreased expression or absence of the glycosylphosphoinositol (GPI) anchor proteins, such as CD55 and CD59, on the erythrocyte and leucocyte membranes. The level of GPI protein expression serves as the basis for classification of cells.

Reference

1. Bain BJ, Barnett D, Linch D, Matutes E, Reilly JT. Revised guideline on immunophenotyping in acute leukaemias and chronic lymphoproliferative disorders. *Clin Lab Haematol* 24, 1-13.

Minutes of the 60th Annual General Meeting held at Kingsgate Hotel, Hamilton on Thursday 26th August 2003 at 7.30am

Chairman
The President (Mr C Kendrick) presided over the attendance of approximately 41 members.

Apologies
That the apologies from Warren Dellow, Michael Legge, Ron McKenzie, Mike Lynch, John Elliot and David Bunker be received.

Proxies
Moved Robin Allen, seconded Ailsa Bunker

That the list of four proxies as read by the Secretary be received.

Carried

Minutes
Moved C Kendrick, seconded R Siebers

That the Minutes of the 59thAnnual General Meeting held on 1 August 2003 be taken as read and accepted as a true and correct record.

Carried

Business arising
Nil.

Remits
Moved J Deans, seconded R Allen

That Policy Decision Number 1 be reaffirmed

Policy Decision No 1 (1971): That all committees and meetings convened under the auspices of the New Zealand Institute of Medical Laboratory Science (Inc) be subject to a standard reference of parliamentary procedure and that this a 'A Guide for Meetings and Organisations' by Renton.

Moved J Deans, seconded T Mace

Policy Decision No 2 (1989): That all persons wishing to undertake any examination offered by the Institute shall at the time of application and the taking of the examination be financial members of the Institute.

Moved K Taylor, seconded R Siebers

That a student membership category be implemented on 1st April 2005.

President's report
Moved C Kendrick, seconded R Hewett

That the President's Report be received.

Carried

Annual report
Moved R Allen, seconded K Taylor

That the Annual Report be received and adopted.

Carried

Financial report
Moved T Mace, seconded T Rollinson

That the Financial Report be received and adopted.

Carried

Election of officers
The following members of Council were elected unopposed:

President	C Kendrick
Vice President	R Allen
Secretary/Treasurer	R Hewett
Region 1 Representative	No candidate
Region 2 Representative	T Mace
Region 4 Representative	K Taylor
Region 5 Representative	A Buchanan

The results of the elections for:

Region 3 Representative	A Thornton	10
	J Wypych	17

Moved, J Deans, seconded R Anderson

That the Election of Officers be approved.

Carried

Awards
The award winners were announced and the awards were presented by the President:

Qualified Technical Assistant Awards

Clinical Biochemistry	Fozia Kan, Diagnostic Medlab
Medical Cytology	Ellana Clendon, Wellington Pathology Laboratory

Haematology	Diane Gillard, Medlab Bay of Plenty
Histology	Greg Senior, Wellington Hospital
Immunology	Kalanithy Ravindran, Diagnostic Medlab
Microbiology	Amy Smith, Medlab Bay of Plenty
Transfusion Science	Charlotte Vanhecke, New Zealand Blood Service
Transfusion Science Blood Products	Alice Tuinukafe, New Zealand Blood Service
Virology	Virginia Wells, Canterbury Health Laboratories
Phlebotomy	Janice Cleland, Medlab Hamilton

Honoraria
Moved R Siebers, seconded R Hewett

That no honoraria be paid.

Carried

Auditor
Moved J Deans, seconded R Allen

That Hilson, Fagerlund and Keyse be appointed as the Institute's auditors.

Carried

General Business
Nil.

Venue for the year 2005 Annual General Meeting

- Christchurch Convention Centre, 14-18 August 2005. Theme - Basic and Beyond - build up from more simple and work up with a lot of inter-discplinary.

Venue for the year 2006 Annual General Meeting
No venue was confirmed.

Meeting closed at 8.15am

NZAP

New Zealand Association of Phlebotomists, SIG of the NZIMLS
Aiming for Excellence

2003 QPT exam

While the over-all results of the first QPT exam were very pleasing, there were several areas where a significant improvement could be achieved by individuals/laboratories for future exams. It was evident that some candidates were sitting an exam for the first time in many years and the following points were noted:

1. A number candidates did not read/interpret the question/s properly.
2. The examination instructions clearly set out by NZIMLS were not followed by approx one third of candidates.
3. There appeared to be poor understanding of a Policy and a Procedure. A policy is "not negotiable" and a procedure is a "step-by-step process".
4. Inadequate organisation of time allocation to question/s with facts listed clearly and concisely - there was a great deal of "in-fill"!
5. Illegible writing in some cases with script hard to follow i.e. some answers were written in the answer book and some on examination paper.

A suggestion was made at the recent meeting of the Auckland Branch of NZAP, to include the topic of "Exam Technique" at the NZIMLS conference in Hamilton in August, on the Phlebotomy Special Interest day. This may be of use to some collection service managers and/or phlebotomists hoping to sit the QPT exam in November this year.

Trish Watt, Training Manager, Diagnostic Medlab, and co-examiner of the 2003 QPT exam.

Patient preparation for a mid stream or clean catch urine - is it necessary?

Laboratories throughout New Zealand have divergent protocols regarding preparation requirements for the "ideal" Mid Stream and/or Clean Catch urine collection. It has historically been considered "best practice" for women to wash the urethral opening and men, the glans, prior to the collection of the middle stream of urine for culture. Elderly, bed-ridden patients and infants provide a "random" specimen because collection of a "sterile mid stream" specimen in these groups is challenging at the best of times.

In reality, many patients neither comply with the "cleansing" process prior to specimen collection nor with catching the "middle part" of the stream. The current literature listed, suggest little or no noticeable difference in culture contaminants from patients who have complied with "ideal" collection requirements and those who have not.

Trish Watt, Training Manager, Diagnostic Medlab, Auckland.

References

1. Lifshitz E, Kramer L. Outpatient urine culture: does collection technique matter? *Arch Intern Med* 2000;160:2357-40.

2. Immergut MA, Gilbert EC, Frensilli FJ, Goble M. The myth of the clean catch urine specimen. *Urology* 1981;17:339-40

3. Leisure MK, Dudley SM, Donowitz LG; Does a clean catch urine sample reduce bacterial contamination? (Letter). *N Engl J Med* 1993;328:289-90

A managers perspective

The QPT, what a great idea, at last those often overlooked ambassadors of our lab could get relevant qualification. Initially I thought we would have several phlebotomists sitting but being cautious some thought they would wait another year.

Gaye and Diana took their own learning in hand armed with a syllabus we discussed who in the lab could explain relevant areas, and then it was up to them. They used the suggested textbooks to good effect and started asking questions of laboratory scientists. The spin off was good as their knowledge increased, and they discussed issues, other phlebs in the team benefited. The knowledge helped make sense of all the rules we have around specimen collection and subsequent analysis.

No amount of reassurance calmed the pre-exam nerves, or the tension on opening the envelope, but then it really was over. Two well deserved excellent results.

As a manager I recommend to other managers to encourage staff to study and sit the QPT as it had such positive benefits for us the laboratory, the candidates, and their colleagues.

Trish Watt, Training Manager, Diagnostic Medlab, Auckland

Exam nerves for the over 40's

My first exam in 25years - the inaugural QPT- Qualified Phlebotomy Technician qualification. An opportunity to increase my knowledge, read around the subject, and thus become a better phlebotomist. Ultimately wear the badge and frame the certificate.

I started reading the recommended textbooks 6 months out, which I discovered gave me plenty of time to learn, forget and then learn again! My grown up children, all last minute crammers, were amazed at my eagerness. However, I found I enjoyed the in-depth study, and things learnt years ago drifted back to the surface.

Exam day inevitably arrived and along with 20 other rather pale looking Phlebs we took our seats, and arranged our biros, barley sugars, and drink bottles. " On your marks, get set, go". And the rest was just a blur.

The euphoria and relief of the next 3 weeks was replaced by intense nail biting. My results were lost in the mail and arrived 3 days after everyone else's.

Gaye Duffill , QPT

Reflections of a QPT

The thought of studying and sitting an exam was terrifying. It's been 25 years since I last did this, but a qualification for my job was something I really wanted. Armed with the recommended textbooks and a syllabus prepared by a dedicated and enthusiastic team of phlebotomy trainers, I started at the beginning of both and worked my way through, sorting out what I knew and what was very vague in my head. With the valuable help of a colleague we collected more information, took tours of the lab, and had brainstorming sessions to see if we could get this down on paper. I started about mid-August which may seem too soon for some, but I needed time to ensure that I knew as much as I could.

On the exam day we said "It's now or never, we are as ready as we will ever be". Our goal was a pass and all we had to do was answer every question. At least, that was what our school teachers always said. At the venue we found the other candidates were feeling just as nervous. This was definitely out of our comfort zone.

The outcome was success and the feeling was, and is, amazing. Now we are just waiting for our badges!

Diana Bell, QPT

Janice Cleland, Medlab Hamilton won the Top Achiever Award for the 2003 QPT exams, which was presented to her at the August 2004 annual Scientific Meeting in Hamilton.

South Island Seminar

Hanmer Springs
The Heritage
Saturday 5th March

The 2005 South Island Seminar will be held in Hanmer Springs. Best known for it's hot pools and exceptional outdoor recreation.

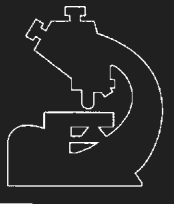
All presenters are most welcome, but as with previous years, first and second time presenters are strongly encouraged.

Post seminar dinner will include live entertainment from the Funky Hot Mama's.

Requests for proffered papers and registration information will be available in early 2005.

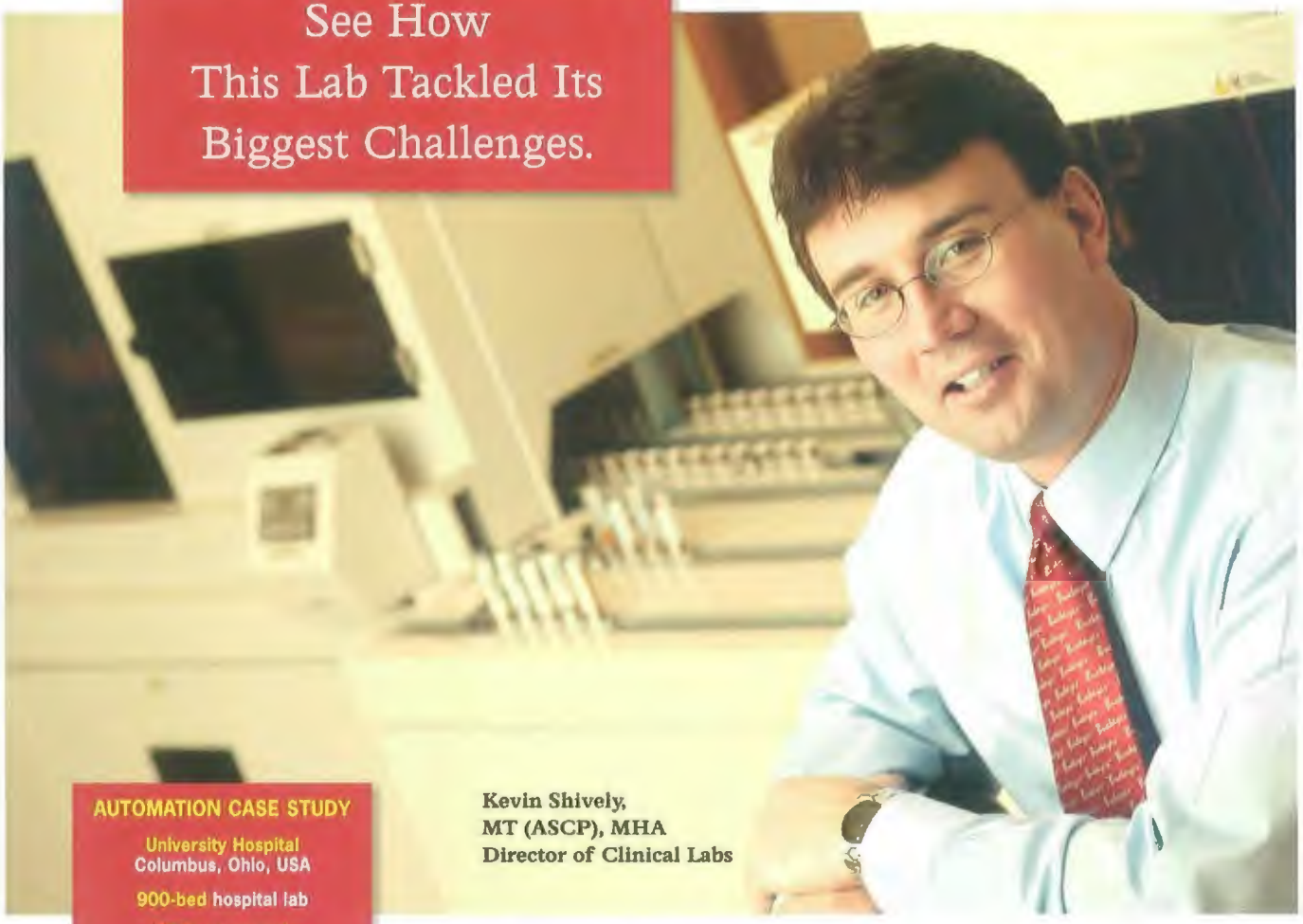
For additional information please contact Kevin Taylor Kevin.Taylor@cdhb.govt.nz or Phone (03) 364 1491.

New Zealand Institute of
Medical
Laboratory
Science



General Chemistry · Immunodiagnostics · Centrifugation · Hematology · Disease Management
Hemostasis · Lab Automation · Data Management · Flow Cytometry · Primary Care

See How This Lab Tackled Its Biggest Challenges.



AUTOMATION CASE STUDY

University Hospital
Columbus, Ohio, USA

900-bed hospital lab

90% automated
for urinalysis, chemistry, haematology and immunoassays

Eliminated 23 of 42 steps
in pre- and post-analytical testing

Reduced turnaround time by 55%

Increased reportable tests by 18%

Decreased worked hours by 23%

Kevin Shively,
MT (ASCP), MHA
Director of Clinical Labs

There's no shortage of challenges for today's clinical laboratory. So when you need to increase efficiency, decrease turnaround time, reduce errors and improve operator safety, turn to Beckman Coulter for innovative automation solutions.

Our automation systems' open architecture provides you with the flexibility to choose instruments from many manufacturers and automate most or all of your testing processes.

And no matter what combination of chemistry, haematology and immunoassay testing you automate, the results are sure to please. Multiple bar-code readers can track patient samples to eliminate errors. And a significant number of steps can be eliminated in pre- and post-analytical testing to maximise efficiency. All of which enables you to improve patient care.

Automation That Fits

If your lab's performance and productivity are falling short of your goals, contact Beckman Coulter or visit us at www.beckmancoulter.com/automation. And we'll show you how easy it is to tackle *your* biggest challenges.

SIMPLIFY · AUTOMATE · INNOVATE

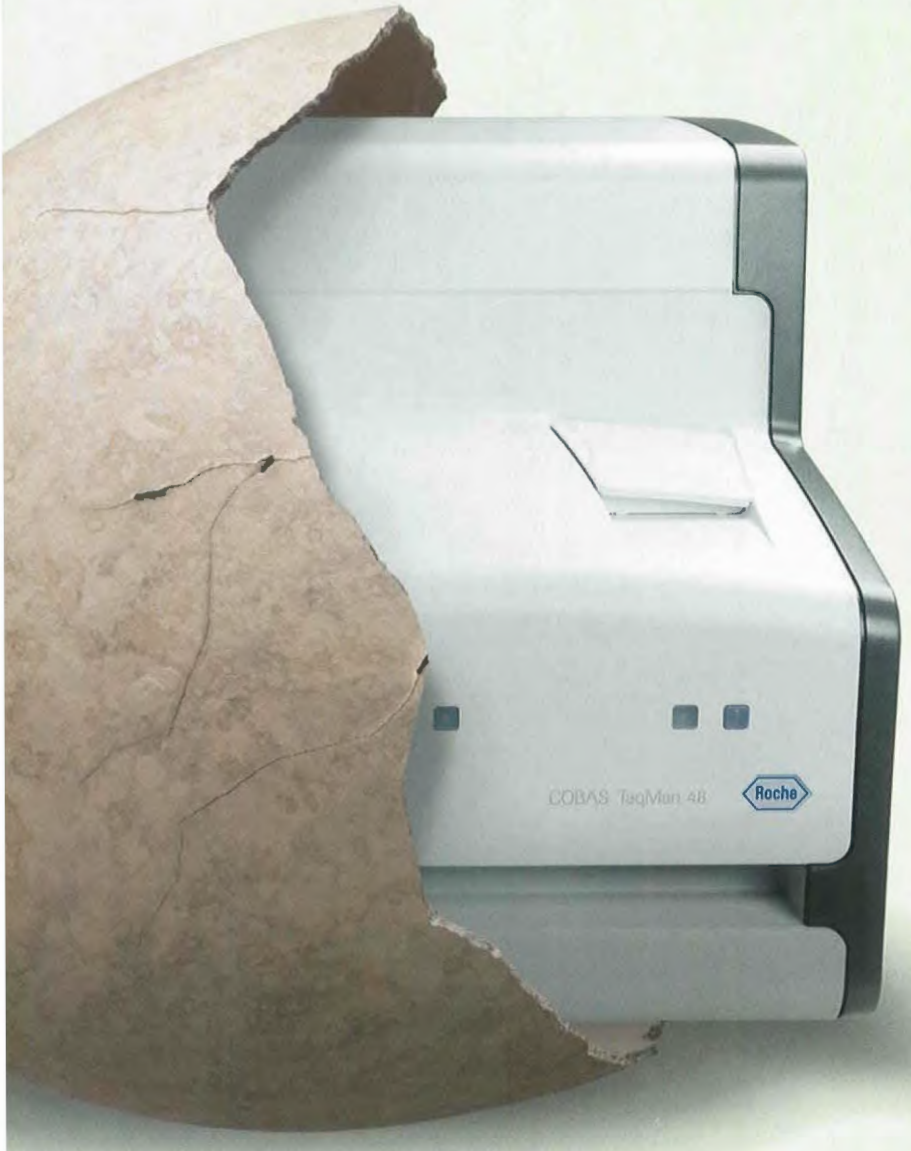
BECKMAN COULTER

For more information contact:
New Zealand: **P** 0800 442 346 **E** sales_aust_nz@beckman.com **W** www.beckmancoulter.com

Roche

Diagnostics

The evolution of PCR



Roche announces the birth of a real-time, high-performance platform that finally makes PCR testing practical for everyday lab use.

The COBAS TaqMan 48 Analyzer combines the superior assay performance labs expect from Roche with **real-time,** rapid quantitative detection of RNA/DNA and a flexible **utility-channel** design that can perform both IVD and user-defined assays. Ready for use now with manual sample preparation; designed for future integration of automated sample preparation capabilities.

Fast. Simple. Flexible. Yours. It's an ideal technological solution for labs looking to evolve.

COBAS
TaqMan
48
Amplify your PCR potential.

Roche Diagnostics New Zealand Ltd
Toll Free: 0800 652 634
Email: elise.bal@roche.com