Laboratory Medicine Research Review™

Making Education Easy

Issue 17 – 2017

In this issue:

Abbreviations used in this issue

ACP = Association of Clinical Pathologists
AML = acute myeloid leukaemia
ASCO = American Society of Clinical Oncology
CVD = cardiovascular disease
EGFR = epidermal growth factor receptor
ELISA = enzyme-linked immunosorbent assay
EPEC = enteropathogenic *Escherichia coli*
HCT = haematopoietic cell transplantation
MIC = minimum inhibitory concentration
NHANES = National Health and Nutrition Examination Survey
PCR = polymerase chain reaction
STEC = Shiga toxin-producing *Escherichia coli*

Welcome to the 17th edition of New Zealand Laboratory Medicine.

In this review, highlights include the influence of having children on life-expectancy, the effect of prior residual leukaemia on disease-free survival after haematopoietic cell transplantation, a non-invasive diagnostic test for male infertility, Māori considerations in relation to biobanking and genomic research, rapid molecular diagnostics for carbapenem-resistant *Acinetobacter* spp., the implementation of recommended extended *RAS* mutation testing, relationships between leukocyte telomere length and biomarkers of cardiovascular disease, and the prevalence of Shiga toxin-producing and enteropathogenic *Escherichia coli* in diarrhoea cases in New Zealand.

We hope you find this issue interesting and look forward to hearing your comments.

Kind regards,

Dr Collette Bromhead
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Payback time? Influence of having children on mortality in old age

Authors: Modig K, et al.

Summary: It is well established that parents live longer than childless individuals. Yet to be elucidated are the underlying mechanisms and how the strength of the association may change with age. This nationwide study in Sweden sought to estimate the association between parenthood and longevity and investigate how it varies with age of the parent and taking into account marital status. Using population registries, all men and women born between 1911 and 1925 and residing in Sweden, and their children, were identified and followed over time. For each calendar year, age-specific risk of death was calculated for those having at least one child and for individuals without children. Risk of death was lower for parents than non-parents: at 60 years of age, life-expectancy was 2 years longer in male parents and 1.5 years longer in female parents. Absolute differences increased with age and were somewhat larger for men than women. The association was stronger for the unmarried, suggesting that social support may be an influencing factor. The gender of the child did not alter the association.

Comment: Introducing a new feature for this issue of Laboratory Medicine Research Review: the wildcard paper. This may be a paper on any aspect of science or medicine that I think readers will find appealing. This quarter I bring you an epidemiological study on how having children affects your mortality! Aptly titled "Payback time?", those of you who are parents will no doubt assume that raising a child is a life-shortening experience. Well, according to this massive study of over 1.4 million Swedish men and women born between 1911 and 1925, that is not the case. Contrary to what I thought, it is apparently well established that parents live longer than non-parents and this paper found a similar inverse association between having a child and death risks in old age, increasing with age of the parent. These death risks were somewhat larger for men than for women and were overall attributed to the apparent increased social support provided by adult children to their elderly parents. This paper included a couple of little gems that made me laugh: "death risk increases with age" (duh) and apparently "marriage has been shown to be more beneficial to men's survival than to women's". Overall, I guess this paper could be of use to those laboratories that are bulk funded by DHBs on a population basis: if your population includes more parents than the national average, you should be funded more per capita because those people will live longer and presumably absorb more pathology tests because of it…!

Reference: J Epidemiol Community Health. 2017 May;71(5):424-30.
Abstract

Prognostic significance of residual acute myeloid leukemia in bone marrow samples taken prior to allogeneic hematopoietic cell transplantation

Authors: Kovach AE, et al.

Summary: The authors of this study investigated associations between signs of residual acute myeloid leukaemia (AML) prior to haematopoietic cell transplantation (HCT) and clinical outcome (leukaemia-free survival) post transplantation. Residual AML was defined by ≥5% aspirate blasts, increased blood blasts, clustered or necrotic blasts on biopsy specimens, and/or leukaemia-associated karyotypic abnormalities. In a cohort of 140 patients, residual AML was identified in 38 (27%) pre-HCT samples. Leukaemia-free survival was significantly shorter in patients with, versus without, morphologic or karyotypic evidence of residual AML (median 7.1 vs 28.3 months, p<0.0001). Multivariate analysis revealed that shorter leukaemia-free survival was independently associated with residual AML, prior relapse, age, high-risk karyotype, and alternate donor source. The association between residual AML and shorter leukaemia-free survival was stronger for intermediate- or favourable-risk AML karyotype (p=0.001) than for secondary or adverse karyotype-risk AML (p=0.04).

Comment: This elegant study retrospectively reviewed the clinicopathologic data for bone marrow samples from 140 adult patients who underwent allogeneic HCT for AML at Massachusetts General Hospital over a 10-year period. They found 38 patients had some evidence of persistent leukaemia in the pre-HCT bone marrow according to morphologic and cytogenetic findings. Rather logically, residual disease is associated with a high likelihood of relapse and short survival following HCT. In particular, residual disease predicts a very poor outcome in patients undergoing HCT with alternate donors and a relatively poor outcome in patients with favourable- or intermediate-risk karyotype AML. Importantly, they found that identification of residual disease should not only rely on the aspirate smear and peripheral blood but also incorporate findings in the core biopsy specimen and bone marrow karyotype. And of course, because it was a retrospective study, this work couldn't determine if additional treatment of patients with residual disease prior to HCT would improve outcomes.

Reference: Am J Clin Pathol. 2017 Jan 1;147(1): 50-9.
Abstract

Metabolic profiling of pregnancy: cross-sectional and longitudinal evidence

Authors: Wang Q, et al.

Summary: The effects of pregnancy on systemic metabolism was investigated by evaluating the detailed metabolic profiles (87 metabolic measures and 37 cytokines) of three population-based cohorts in Finland comprising 4260 women (24–49 years, 322 pregnant) and comparing the circulating molecular concentrations of pregnant women with non-pregnant women. Longitudinal metabolic changes associated with pregnancy status were also assessed by re-measuring metabolic profiles in 583 women 6 years later. Marked increases in all lipoprotein subclasses and lipids were observed for pregnant versus non-pregnant women, with the largest increases reported for intermediate-density, low-density and high-density lipoprotein triglyceride concentrations. Major differences in the levels of many fatty acids and amino acids were also evident. Increased concentrations of low-grade inflammatory marker glycoprotein acetyls, higher concentrations of interleukin-18 and lower concentrations of interleukin-12p70 were also observed for pregnant women. The cross-sectional association pattern was replicated in women who were not pregnant at baseline but pregnant 6 years later (or vice versa); it was also consistent across the three cohorts. Overall, changes in metabolic measures during pregnancy increased in magnitude across the three trimesters.

Comment: This paper annoyed the dickens out of me. It's one of those smug papers in a high-impact journal that will probably be forever cited by all who follow because it was the first to say what "normal results" should be. And they didn't even have to put the hard work into recruiting the 4260 women (322 who were pregnant) who took part in the study as they were already part of other larger population-based birth cohorts in Finland (similar to Otago University's famous "Dunedin Cohort"). Then, the samples they tested went through a huge nuclear magnetic resonance metabolomics platform that analysed 124 biomarkers, including 87 metabolic measures and 37 cytokines, and generated so much data that when I tried to look at the results, they kept on referring me to further supplementary files. REALLY annoying. So what did they find from this mountain of information? Funnily enough, that the metabolic effects of pregnancy are exceptionally large, gradually increase across the trimesters, and generally normalise within 3–6 months postpartum. Interestingly however, the pattern of metabolic changes to fatty acids, amino acids and inflammatory markers (C-reactive protein, etc.) during pregnancy has a strong resemblance to the one caused by the combined oral contraceptive pill. Progestin-only contraceptives have no such effects. This implies that oestrogen plays a key role in the regulation of maternal metabolism and is to blame therefore for the adverse side effects some women experience when taking the combined oral contraceptive pill. And that's all I have to say about the paper.

Reference: BMC Med. 2016 Dec 13;14(1):205.
Abstract

Preclinical evaluation of a TEX101 protein ELISA test for the differential diagnosis of male infertility

Authors: Korbakis D, et al.

Summary: The authors previously validated TEX101, a cell membrane protein expressed exclusively by testicular germ cells and shed into seminal plasma, as a biomarker for the differential diagnosis of azoospermia. They used mass spectrometry, size-exclusion chromatography, ultracentrifugation, and immunohistochemistry to characterise TEX101 protein as an analyte in seminal plasma and subsequently developed an enzyme-linked immunosorbent assay (ELISA) for TEX101. In this study, the authors sought to assess the performance of their TEX101 ELISA in fertile, subfertile, and infertile men. Analysis of 805 seminal plasma samples showed that TEX101 was present mostly in a free soluble form and that its small fraction was associated with seminal microvesicles. Median values for TEX101 were 5436 ng/mL in healthy, fertile pre-vasectomy men (n=64), 4967 ng/mL in men with unexplained infertility (n=277), 450 ng/mL in cases of oligospermia (n=270), and 0.5 ng/mL in men with azoospermia (n=137). There were undetectable levels of TEX101 (≤0.5 ng/mL) in fertile post-vasectomy men (n=57), men with Sertoli cell-only syndrome (n=13) and men with obstructive azoospermia (n=36). To distinguish between pre- and post-vasectomy men, a cut-off value of 0.9 ng/mL was found to provide 100% sensitivity at 100% specificity. The possibility to eliminate the need for most diagnostic testicular biopsies by differentiating between non-obstructive and obstructive azoospermia was highlighted by combining the results for TEX101 (cut-off 0.9 ng/mL) and epididymis-specific protein ECM1 (cut-off 2.3 µg/mL), which provided 81% sensitivity at 100% specificity. Furthermore, in cases of non-obstructive azoospermia a TEX101 cut-off value of ≥0.6 ng/mL provided 73% sensitivity at 64% specificity for predicting sperm or spermatid retrieval

Comment: At last, I hear you cry, here is one for the fertility scientists! This is a really solid piece of work and adds greatly to the field. First, this group generated their own monoclonal antibody to TEX101, a cell membrane protein exclusively expressed by testicular germ cells and shed into seminal plasma. Then, they worked up an ELISA with this monoclonal antibody using seminal plasma, a promising but unconventional fluid for clinical diagnostics. It's an intriguing sample because testis-specific bio-blood-testis and blood-epididymis barriers ensure that semen and seminal plasma remain the only viable fluids for the non-invasive diagnosis of male infertility. Indeed, this study validated the TEX101 ELISA as a clinical test to evaluate vasectomy success, stratify azoospermia forms and subtypes, and predict the success of sperm retrieval in non-obstructive azoospermia patients. From the patient's point of view, this is an important new assay as it may save many from testicular biopsies. Bravo!

Reference: BMC Med. 2017 Mar 23;15(1):60.
Abstract

Key informant views on biobanking and genomic research with Maori

Authors: Hudson M, et al.

Summary: The Te Mata Ira project was a 3-year research project that explored Māori views on biobanking and genomic research in order to address Māori concerns over the collection and use of human tissue and for the development of culturally appropriate guidelines. Māori views on the subject were initially identified through key informant interviews and workshops with Māori. Informed by these views, further interviews and workshops were conducted with Māori and non-Māori key informants (Indigenous Advisory Panel members and science communities) to explore key issues surrounding Māori participation in biobanking and genomic research. The following key considerations were identified by Māori key informants: (1) the tension for Māori between previous well-publicised negative experiences with genomic research and the potential value for whānau and communities as technologies develop, (2) protection of Māori rights and interest, (3) focus on Māori health priorities, (4) control of samples and data, (5) expectations of consultation and consent and (6) a desire for greater feedback and communication. A need to increase Māori participation in the governance of genomic research and biobanking initiatives was noted by Māori and non-Māori key informants, who also recognised that increasing the level of transparency and accountability in relation to these activities is necessary in order for Māori communities to feel that their whakapapa, rights and interests are being appropriately protected.

Comment: This paper described the findings from a project that was aimed at plugging perceived gaps in the previously published *Te Ara Tika-Guidelines on Maori Research Ethic: A framework for researchers and ethics committee members*. Using qualitative research techniques (interviews, focus groups), it addresses the fact that there are a number of "informal biobanks" containing research-based tissue collections which have samples that have been consented for future use. But with no register there is no way of knowing how many samples are in storage, how many have been provided by Māori participants and how long they have been stored, nor the quality systems underlying the process of notifying participants of particularly genomic results that may affect their wider whānau. The researchers found that communities are becoming more critical about the nature of participation and expectations of researchers, particularly in light of well publicised past poor experiences such as the "warrior gene" work done in NZ. As far as solutions, they are not radical and include medical researchers building a higher level of engagement and consultation for processes of consent, communication of what's in it for the participants and their whānau, and allowing ongoing participant control over samples and data, including the robustness and interpretation of genomic research methods. However, this paper does state that there is a wide range of views in the Māori community and it stops short of giving a utilitarian checklist that could be used by frazzled researchers and ethical review committees who are just trying to do the right thing.

Reference: N Z Med J. 2016 Dec 16;129(1447):29-42.
Abstract

Independent commentary by Dr Collette Bromhead, Massey University

As well as academic teaching and research, Collette maintains roles with IANZ and the National Cervical Screening Program, latterly as part of the Technical Reference Group advising the ministry on the implementation of HPV Primary Screening for Cervical Cancer.

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Informing antibiotic treatment decisions: evaluating rapid molecular diagnostics to identify susceptibility and resistance to carbapenems against *Acinetobacter* spp. in PRIMERS III

Authors: Evans SR, et al.

Summary: Two rapid molecular diagnostics platforms, polymerase chain reaction (PCR) combined with electrospray ionization mass spectrometry and molecular beacons, were evaluated for the detection of genes conferring resistance/susceptibility to carbapenems in *Acinetobacter* spp. A panel of 200 *Acinetobacter* spp. isolates from locations worldwide was assembled, using minimum inhibitory concentrations (MICs) for each strain as the reference standard to define susceptibility or resistance. The absence or prevalence of the most relevant and prevalent carbapenemase genes was investigated, i.e. for the beta-lactamase genes $bla_{OXA\text{-}23}$, $_{OXA\text{-}24/40}$, $_{OXA\text{-}58}$, $_{NDM}$, $_{KPC}$, $_{VIM}$, and $_{IMP}$. Predictive values for susceptibility and resistance were estimated based on the probability of the MIC result indicating susceptibility or resistance and as a function of the prevalence of susceptibility. A total of 98 isolates (49%) were carbapenem resistant (defined by either resistance or intermediate resistance to imipenem) according to interpretation of the MICs. Susceptibility sensitivities for imipenem were 82% (95% CI 74–89%) for PCR-mass spectrometry and 92% (95% CI 85–97%) for molecular beacons. Resistance sensitivities for imipenem were 95% (95% CI 88–98%) and 88% (95% CI 80–94%) for PCR-mass spectrometry and molecular beacons, respectively.

Comment: Ah, that old chestnut: shall we genotype to rapidly determine antimicrobial susceptibilities or culture and determine them phenotypically, according to the standards? This is the third in a series of studies on "platforms for rapid identification of multidrug-resistant gram-negative bacteria and evaluation of resistance studies". An overly long title so they could get the acronym "PRIMERS" out of it. Sigh. So the target this time is the often mis-identified *Acinetobacter* spp. The authors state that due to rising antimicrobial resistance, carbapenems are the cornerstone of therapy for serious infections due to *Acinetobacter* spp. So they employed two rapid molecular diagnostic platforms: PCR combined with mass spectrometry and molecular beacons for detecting *bla* genes associated with resistance to carbapenemases. Tested against a panel of 200 isolates, they correctly identified resistance in 88–94% of the samples compared to phenotypic methods. However, in a clinical laboratory where carbapenem resistance is low, the accuracy of this method may fall.

Reference: J Clin Microbiol. 2016 Dec 28;55(1):134-144.
Abstract

How close are we to standardised extended RAS gene mutation testing? The UK NEQAS evaluation

Authors: Richman SD, et al.

Summary: *KRAS* mutation testing has been a compulsory prerequisite for metastatic colorectal cancer treatment with anti-epidermal growth factor receptor (EGFR) therapies for several years, with *KRAS* mutation status in exon 2 (codons 12 and 13) used to predict response. However, recent analyses have supported the introduction of extended *RAS* testing to include more mutation hotspots in *KRAS* and also *NRAS* testing. Thus, in 2014 in the UK the Association of Clinical Pathologists (ACP) issued guidelines recommending as a minimum the testing of *KRAS* codons in exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) and also the same *NRAS* codons in exons 2 and 3. Similarly, the American Society of Clinical Oncology (ASCO) published 2015 updated guidelines recommending extended testing for mutation status in both *KRAS* and *NRAS* codons 12, 13, 59, 61, 117 and 146. Following the release of the new recommendations in the UK, the authors of this study assessed the range of *RAS* codons being routinely tested across four UK National External Quality Assessment Service for Molecular Genetics colorectal cancer External Quality Assessment schemes during the period 2014–2016 in up to 110 UK and international laboratories. *KRAS* codons 12, 13 and 61 were routinely tested by at least 85% of laboratories and across the four schemes an increasing number of laboratories routinely tested *KRAS* codons 59, 117 and 146. Although fewer laboratories tested for *NRAS*, the pattern of extended testing was similar. However, the fact that only 36.1% and 24.1% of participating laboratories met the ACP and ASCO guidelines, respectively, is of concern since inadequate testing results in patients being unnecessarily exposed to inappropriate anti-EGFR treatment that may be harmful to them.

Comment: This very interesting paper is a sign of the times and the onward march of genomics in personalised treatment of colorectal cancers. While it has been well established that patients with mutations in exon 2 (codons 12 and 13) of *KRAS* fail to respond to anti-EGFR therapy, the list of recommended codons to test has exploded (particularly after ASCO's update last year) and I can see it continuing to do so. Little wonder then that this UK National External Quality Assessment Service survey found only 24.1% of 108 worldwide laboratories performing *RAS* mutation testing were meeting the ASCO guidelines and 36.1% were meeting the less stringent UK ACP guidelines. With only two FDA-approved mutation screening kits (Therascreen RGD and the Roche cobas *KRAS* mutation testing kit) both confined to codons 12 and 13, the shift to next-generation sequencing is notable among laboratories attempting to grow the span of genotyping they can offer. However, the need is great with studies suggesting that there are still between 14.7% and 17% of patients with an additional *RAS* mutation, indicating a larger than necessary patient cohort subjected to detrimental effects of anti-EGFR therapies that would not have been prescribed had an extended *RAS* panel been incorporated into practice.

Reference: J Clin Pathol. 2017 Jan;70(1):58-62.
Abstract

Leukocyte telomere length in relation to 17 biomarkers of cardiovascular disease risk: a cross-sectional study of US adults

Authors: Rehkopf DH, et al.

Summary: Leukocyte telomere length is significantly and negatively associated with cardiovascular disease (CVD) risk. In this study, the authors investigated the relationship between leukocyte telomere length and known metabolic and pro-inflammatory markers and CVD risk factors. Using data from adults aged 20–84 years (n=7252) from the US 1999–2002 National Health and Nutrition Examination Survey (NHANES), associations between leukocyte telomere length and 17 cardiovascular biomarkers were evaluated, including lipoproteins, blood sugar, circulatory pressure, pro-inflammatory markers, kidney function, and adiposity measures. Statistical adjustments were made for immune cell type distributions and the effects of age, race/ethnicity, gender, education, and income on associations were assessed. A one unit difference in the following biomarkers was significantly associated with kilobase pair differences in leukocyte telomere length: body mass index, waist circumference, percentage of body fat, high-density lipoprotein cholesterol, triglycerides, pulse rate, C-reactive protein, and cystatin C. Using clinical cut-points, additional significant associations were found between leukocyte telomere length and insulin resistance, systolic blood pressure, and diastolic blood pressure. Age, race/ethnicity, gender, education, and income had little impact on the associations.

Comment: Another reanalysis from a previously collected large dataset (NHANES), this time looking at whether telomere length in white cells was related to CVD risk factors. A strength is that the population studied was aged 20–85, so a wide range of telomere length variability could be expected naturally. For me the main bonus was that this study could put to bed the question about the significance of telomere length in CVD risk that had previously been raised by smaller studies in confined population groups. Because let's face it, the results weren't startling and just confirmed what we already suspected: telomere length in white cells is associated with risk factors across many different physiological systems and cannot be pinpointed as a direct cause of CVD. Job done.

Reference: PLoS Med. 2016 Nov 29;13(11):e1002188.
Abstract

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Prevalence of Shiga toxin-producing and enteropathogenic *Escherichia coli* marker genes in diarrhoeic stools in a New Zealand catchment area

Authors: Thomas RR, et al.

Summary: This study assessed the prevalence of the gastrointestinal pathogens Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) in cases of diarrhoea in New Zealand, which is currently unknown due to a lack of EPEC screening and use of Sorbitol-MacConkey (SMAC) agar in faecal screening. A multiplex TaqMan quantitative PCR assay was used to detect *stx1*, *sxt2* and EPEC (*eae*) gene markers in DNA extracted from 522 pre-enriched diarrhoeic samples. A total of eight (1.5%) were positive for *stx1*/*sxt2* and 23 (4.4%) were positive for *eae*. Of the *stx*$^+$ samples, six (75%) were uncommon non-O157 serotypes and the remaining two (25%) were positive for both O103 and O157 STEC somatic antigens.

Comment: This is the type of study that a NZ reference laboratory or even the local tertiary hospital laboratory should be funded to do: to occasionally evaluate the contribution of STEC and EPEC to diarrhoea cases in the hospital and community. However, this work seems to come increasingly under the heading university research, funded through usually competitive means. The upshot, though, is that the testing is not then conducted in an IANZ accredited laboratory. Although the methods in this paper were extremely robust, this gives clinical laboratory staff reason to pause and critically evaluate before they could implement such a method into their clinical laboratory. Anyway, it is a nice study that showed a comparable prevalence of *stx*$^+$ and *eae*$^+$ *E. coli* to other high-income countries and may spur revision in the diagnosis of these pathogens in the future.

Reference: J Clin Pathol. 2017 Jan;70(1):81-84.
Abstract

CONGRATULATIONS TO

Dr Robin Rund

who won an iPad mini 3 by taking part in our recent subscriptions update promotion. Robin is an Anaesthetist at the Bay of Plenty District Health Board.

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