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- Surgical Safety Checklist compliance and specimen labelling errors
- Cost minimisation in lung cancer molecular pathology testing
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- Unusual case of lymphogranuloma venereum
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- Low-cost population screening of silent MI

Abbreviations used in this issue

ANA = antinuclear antibody
CIN3+ = cervical intraepithelial neoplasia grade 3 or worse
CMR = cardiovascular magnetic resonance
HPV = human papillomavirus
LGE = late gadolinium enhancement
LGV = lymphogranuloma venereum
MI = myocardial infarction
NT-proBNP = amino-terminal pro-brain natriuretic peptide
PCR = polymerase chain reaction
TTF1 = thyroid transcription factor 1

Welcome to the 16th issue of New Zealand Laboratory Medicine Research Review. In this issue, highlights include improved identification of the causes of diarrhoea in children using quantitative PCR, the latest European guidelines on Mycoplasma genitalium infections, a limited role for serial ANA tests, a new approach introduced in New Zealand to improve compliance with the World Health Organization Surgical Safety Checklist, strategies to reduce costs in lung cancer molecular pathology testing, data that shows extending the cervical cancer screening interval may be warranted, quality metric data trends for chromosome microarrays, clinical practice guidance on investigating and managing hyperferritinaemia, and a novel means to screen for silent MI in patients with type 2 diabetes. We hope you find this issue interesting and look forward to hearing your comments.

Members of the NZIMLS CPD programme can now use Laboratory Medicine Research Review for CPD. A claim of 2 points is available for reading an issue of this publication and writing a synopsis for audit purposes.

I wish you all a safe and happy Christmas and New Year.

Kind regards,
Dr Collette Bromhead
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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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Merry Christmas and a healthy, happy 2017!

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www.researchreview.co.nz
Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study

Authors: Liu J, et al.

Summary: The Global Enteric Multicenter Study (GEMS) was a large case-control study of moderate to severe diarrhoea in children aged <5 years in Africa and Asia that used microbiological methods, including culture, enzyme-linked immunosorbent assay and reverse-transcriptase polymerase chain reaction (PCR) to identify infectious causes. This re-analysis sought to reassess the causes of diarrhoea using quantitative real-time PCR to test for 32 enteropathogens in stool samples from cases and matched asymptomatic controls. Of 5304 sample pairs tested, the total pathogen-attributable diarrhoeal burden was 89.3% with quantitative PCR compared with 51.5% in the original GEMS analysis. Incidence of infection was highest for most pathogens; by about five times for adenovirus 40/41, by about two times for Shigella spp or enteroinvasive Escherichia coli and Campylobacter jejuni or C. coli, and by about 1.5 times for heat-stable enterotoxin-producing E. coli. The top six attributable pathogens accounted for 77.8% of all attributable diarrhoea; in descending order they were Shigella spp, rotavirus, adenovirus 40/41, heat-stable enterotoxin-producing E. coli, Cryptosporidium spp, and Campylobacter spp. Applying model-derived cut-offs, 42.5% of cases had one causative pathogen detected and 38.9% had two or more (most commonly involving Shigella spp and rotavirus).

Comment: Public health interventions rely on estimates of pathogen-specific burden for prioritisation. However, the sensitivity of the test method used for such surveys can substantially affect burden estimates. This case-controlled study used a custom TaqMan array card (thermo Fisher) that allowed for standardised quantitative real-time PCR testing of 32 enteropathogens. Rather complicated epidemiological statistics were used to determine a Cq cut-off for each pathogen adjusted for the high prevalence of asymptomatic carriage. For most pathogens, the quantitative PCR-derived attributable incidence surpassed the original estimate, and the authors surmised that targeted interventions, such as vaccination development, could have larger public health benefits than previously projected. This was probably the point of this paper — to build a case for more funding to develop new vaccines. As a diagnostic strategy, quantitative PCR will inherently function less well for pathogens that are shed with high frequency, in high quantities and for an extended duration in the absence of diarrhoea. Unfortunately, this study did not provide speciation and subtyping, which would be required for the presumably desired vaccine development. I’m guessing there is another study in the works then?


2016 European guideline on Mycoplasma genitalium infections

Authors: Jensen JS, et al.

Summary: Mycoplasma genitalium infection is associated with non-gonococcal urethritis in men, especially non-chlamydial non-gonococcal urethritis (accounting for 10-35% of cases), and cervicitis and pelvic inflammatory disease in women. Diagnosis is achieved with nucleic acid amplification testing, which is indicated in the presence of symptoms or high-risk sexual behaviour (asymptomatic infections are frequent). Following diagnosis, an assay for macrolide resistance should be performed, if available. Detection of M. genitalium and recent sexual contact (previous 3 months) with an infected individual are indications for treatment. In macrolide susceptible infections, azithromycin achieves a cure rate of 85-95% compared with 30-40% with doxycycline, although the latter does not increase resistance. Widespread use of azithromycin 1 g single dose without test of cure is the most likely cause of drastically declining overall cure rates due to macrolide resistance (an extended course appears to have a higher cure rate). For uncomplicated macrolide susceptible M. genitalium infection, the recommended first-line treatment is oral azithromycin 500 mg on day 1, then 250 mg on days 2-5, or oral josamycin 500 mg three times daily for 10 days. Moxifloxacin 400 mg once daily for 7-10 days can be used as second-line treatment or in macrolide resistant infection. Third-line treatment options include doxycycline 100 mg twice daily for 14 days or oral pristinamycin 1 g four times daily for 10 days. The recommended treatment of complicated M. genitalium infection (pelvic inflammatory disease, epididymitis) is moxifloxacin 400 mg once daily for 14 days.

Comment: Firstly, my congratulations to Dr Massimo Giola from BOP DHB who gave a very elegant presentation on these important new guidelines at the recent NZ Sexual and Reproductive Health and Rights conference held in Wellington, timed brilliantly for just before the November earthquake swarm kicked off. As he pointed out, the unassuming M. genitalium has barely made it onto the healthcare radar in NZ but threatens to take the prize for the most antibiotic resistant genitourinary pathogen in the country. This is a problem since we have only patchy availability of nucleic acid amplification testing for M. genitalium and no routine availability of macrolide susceptibility genotyping indicated by this guideline. However, I have recently successfully trialled the SpeeDx Mycoplasma genitalium Resistance Plus kit which generates mutations in the 23S rRNA gene associated with resistance to macrolides, and I believe that Canterbury Health Laboratories are now offering this assay. Unfortunately, in the study on moxifloxacin, the recommended alternative therapy to azithromycin, is also losing its efficacy with many strains acquiring the gyrA mutation associated with quinolone resistance. Furthermore, third-line pristinamycin is quoted as giving a cure rate of approximately 90%...how long until that falls too?


The concordance of serial ANA tests in an Australian tertiary hospital pathology laboratory

Authors: Lee AF, et al.

Summary: The authors of this study performed a retrospective analysis of serial antibacterial antibody (ANA) testing between 2008 and 2014 at a single pathology laboratory in Australia to assess its statistical reliability. Of 6149 ANA requests, 776 were repeat requests in 599 patients. The authors reported reasonable repeatability for titre and pattern with height-titrated ANA (>1:640) compared with poor repeatability for low-titrated ANA (1:40 or 1:80). Overall, the authors concluded that there is little benefit in serial ANA testing except in cases of an initial low-titrated ANA and a subsequent clear change in the patient’s clinical picture.

Comment: A wise mentor once told me that ANA’s were more art than science. This interesting paper would tend to support that view, especially for positive results at low titres. ANA tests are some of the more frequently requested tests for diagnosis of autoimmunity. Although they are used primarily as diagnostic blood tests, multiple requests on the same patient continue to be encountered in the laboratory. This would seem a poor use of laboratory resources given that this paper showed low titre staining patterns are almost random on serial tests when compared to the first-obtained ANA pattern. Laboratory measures such as having timeframe policies for retests, and delta checks for previous results can reduce testing and highlight discrepancies. For the persistent over-testers, you could always go with the alternative Rorschach test (https://en.wikipedia.org/wiki/Rorschach_test).

Improved compliance with the World Health Organization Surgical Safety Checklist is associated with reduced surgical specimen labelling errors

Authors: Martis WR, et al.

Summary: This retrospective New Zealand study assessed the rate of surgical specimen labelling errors within the Auckland District Health Board before and after the introduction of a new approach to administering the surgical safety checklist using wall-mounted charts for each domain. The new approach was associated with improved compliance with the Sign Out domain and a reduction in the rate of surgical specimen labelling errors from 19 in 4760 specimens (rate 3.99/1,000) in the 6 months before to eight in 5065 specimens (rate 1.58/1,000) in the 6 months after the change in surgical safety checklist administration (p=0.0225).

Comment: Specimen labelling errors may have serious consequences. This important local study shows that improved compliance with the World Health Organization Surgical Safety Checklist was associated with a significant improvement in the frequency of administration of the Sign Out domain from 22% to 84% of cases, a phase of care that requires explicit review of the number, nature and labelling of specimens. This is turn led to a significant reduction in specimen labelling errors at Auckland District Health Board. Strengthening the communication of the process and spreading the responsibility for each stage of compliance to the anaesthesia, surgery and nursing teams respectively contributed to this improvement. As the authors point out, adopting a checklist “in name” is not sufficient to reap its potential benefits; it actually has to be used!


Could molecular pathology testing in lung cancer be more cost-effective?

Authors: Walsh K, et al.

Summary: In order to explore different strategies that might reduce laboratory service costs associated with non-small cell lung carcinoma, data from an audit of molecular pathology testing in the South East Scotland Cancer Network was analysed, including an assessment of thyroid transcription factor 1 (TTF1) expression as a negative predictor for EGFR mutations. TTF1 immunohistochemistry demonstrated a 99% negative predictive value for EGFR mutations. Compared with reflex testing of all non-squamous non-small cell lung carcinomas for EGFR and ALK mutations, a 7.5% reduction in costs could be achieved by limiting testing to only those who might be considered for targeted treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors or anaplastic lymphoma kinase (ALK) inhibitors. However, with a serial testing model based on the mutually exclusive relationship between EGFR, KRAS and ALK mutations, a 32.7% reduction in costs was possible. If TTF1 is validated as a negative predictive biomarker, further savings could be made (total cost reduction of 37.5%).

Comment: The existence of this paper is reflective of both the expensive nature of molecular oncology testing and the fact that most clinical laboratories are experiencing increasing pressures to reduce costs. This clinical audit of lung cancer pathology requests trialed four models of testing algorithms and found the most cost saving approach was to test all non-small cell lung carcinoma patients with immunohistochemistry for TTF1 and ALK rearrangements, where only TTF1-positive, ALK-negative samples would have KRAS mutation analysis and then only those with no KRAS mutations would be tested for EGFR mutations. The disadvantages include an extended turnaround time compared with reflex testing, and the risk of exhausting the sample when specimen size and thus DNA yield is limiting. Although promising, there is too little data on the association between TTF1 immunohistochemistry and EGFR to fully support its use as a negative predictor for EGFR mutations. As ever, further work is needed.


Safety of extending screening intervals beyond five years in cervical screening programmes with testing for high risk human papillomavirus: 14 year follow-up of population based randomised cohort in the Netherlands

Authors: Dijkstra MG, et al.

Summary: In the population-based POBASCAM trial in the Netherlands, 43,339 women aged 29-61 years with a negative human papillomavirus (HPV) and/or negative cervical cytology test were randomised to HPV and cytology co-testing (intervention) or cytology only (control) comprising of three screening rounds, one every 5 years. The cumulative incidences of cervical cancer and cervical intraepithelial neoplasia grade 3 or worse (CIN3+) were similar among HPV-negative women in the intervention group after three rounds of screening (0.09% and 0.56%, respectively) and among women with negative cytology in the control group after two rounds (0.09% and 0.69%, respectively). Risk ratios for cervical cancer and CIN3+ were not significant. In HPV-negative women, CIN3+ incidence was 72.2% lower among those aged 40 years or more versus younger women (p<0.001). No significant association between cervical cancer incidence and age was reported. Among HPV-positive women with negative cytology, HPV 16/18 genotyping, and/or repeat cytology, CIN3+ incidence was 10.4 times higher than among HPV-negative women. The authors concluded that these long-term data support an extension of the cervical screening interval beyond five years for women aged 40 years and older.

Comment: I couldn’t let an issue of the Review go past without including a paper on cervical screening and HPV testing! This study reports on a 14-year long follow-up of the large Dutch POBASCAM study, which compared HPV and cytology co-testing with cytology only, and used 5 yearly screening intervals. The study findings support what is already known about the long-term protective effect of a HPV-negative test, and the limited value of primary HPV and cytology co-testing. Additionally, the data confirms that in women at least 40 years old, a 10-year screening interval confers good safety for both cervical cancer and CIN3+. This is pretty good news for women in the Netherlands, who in 2017 will move to an HPV primary screening programme with exactly these 10-year intervals for 40+ women. This approach is termed “risk-based screening” and is not being considered in Australia or New Zealand, with both following the Netherlands into the land of HPV primary screening in 2017 and 2018, respectively, but with 5-year intervals over all age ranges. Whew.


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How should hyperferritinaemia be investigated and managed?

Authors: Ong SY, et al.

Summary: Hyperferritinaemia may be due to true iron overload or a result of conditions such as excess alcohol intake, inflammation and non-alcoholic fatty liver disease. It is therefore important to identify the cause of hyperferritinaemia. Establishing whether serum ferritin is raised should take into account body mass index, gender and age. An algorithm is provided for clinicians to use in clinical practice to carry out appropriate investigations and management.


Abstract

A novel and practical screening tool for the detection of silent myocardial infarction in patients with type 2 diabetes

Authors: Swoboda PP, et al.

Summary: Silent myocardial infarction (MI) is a common occurrence in type 2 diabetes patients. The most validated technique to investigate silent MI is late gadolinium enhancement (LGE) by cardiovascular magnetic resonance (CMR). Since this method is time-consuming, costly, and requires administration of intravenous contrast, the authors of this study aimed to develop a low-cost population screening tool to identify type 2 diabetes patients at highest risk of silent MI, validated against the CMR reference standard. Electrocardiogram, echocardiography, biomarker assessment, and CMR at 3.0T were performed on 100 asymptomatic type 2 diabetes patients with no history of cardiovascular disease. Global longitudinal strain from two- and four-chamber views was measured using feature tracking. Silent MI was detected in 17 patients by LGE on CMR, only four of which had Q waves on electrocardiogram. Older age (65 vs 60, p<0.05), lower E/A ratio (0.75 vs 0.89, p=0.004), lower global longitudinal strain (-15.2% vs -17.7%, p=0.004), and higher amino-terminal pro brain natriuretic peptide (NT-proBNP) (106 ng/L vs 52 ng/L, p=0.003) were significantly associated with silent MI. A risk score combining these four factors had an area under the receiver operating characteristic curve of 0.823 (0.734-0.892), p<0.0001. For a score >3/5, sensitivity for silent MI was 82% and specificity 72%.

Reference: J Clin Endocrinol Metab. 2016 Sep;101(9):3316-23.

Abstract

Chromosome microarray proficiency testing and analysis of quality metric data trends through an external quality assessment program for Australasian laboratories

Authors: Wright DC, et al.

Summary: Quality control metrics are needed to minimise false-positive and false-negative results of chromosome microarrays for investigating chromosome and gene copy number mutations. The authors of this study reported on an external chromosome microarray proficiency testing programme for Australasian laboratories that evaluated analytical accuracy, result interpretation, report completeness, and laboratory performance data. Between 2009 and 2014, nine proficiency test samples with data from up to 23 laboratories demonstrated 30-100% analytical accuracy, 32-96% correct interpretation and 30-92% report completeness. Between 2007 and 2014, laboratory performance metrics indicated an overall mean success rate of 99.2% and abnormality rate of 23.6%. During this period, reporting times decreased from >90 days to <35 days for normal results and from >102 days to <35 days for abnormal results. Data trends showed a significant positive correlation for report completeness and reporting times. It remains unclear whether overall improvement in laboratory performance was a result of participation in the programme or from increasing laboratory experience over time.

Comment: The need for external quality assurance was underlined in this study by the initially alarming data from the pilot in 2009 where only three out of 10 laboratories correctly identified and reported a male with no abnormality. However, interpretation performance improved over time, but considerable variability persisted in report completion, with data such as sample identifiers and date received, microarray design and genome build often left incomplete. Median reporting times have improved for both normal and abnormal results. There was a steady increase in the number of laboratories and the number of microarray tests, reflecting the growth of molecular genetics in diagnostic pathology overall. The migration of what was originally a research tool into the clinical laboratory has been technically challenging and has necessitated the establishment of quality control metrics for as many aspects of laboratory practice associated with chromosome microarray testing and analysis. Conceptually, this has been the case for a number of molecular and genetic technologies and will continue to be so, especially given the increasing diagnostic utility of Next Generation Sequencing for both genetic and infectious diseases.


Abstract

Lymphogranuloma venereum presenting with erythema nodosum

Authors: Borsje A, et al.

Summary: Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by Chlamydia trachomatis serovar L1, L2 or L3 that is endemic in tropical and subtropical regions of the world, although outbreaks have been reported since 2003 among men who have sex with men in Europe and North America, mostly (80%) in HIV-positive men. This paper described the case of a 30-year-old HIV-negative man in the Netherlands with inguinal lymphadenopathy. He denied having sex with men. His prior medical history was unremarkable. The paper described the case of a 30-year-old HIV-negative man in the Netherlands with inguinal lymphadenopathy. He denied having sex with men. His prior medical history was unremarkable. The authors proposed that this highlights the need to consider a diagnosis of LGV outside the usual context.


Abstract


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