Results: As a cohort, there was no significant difference in post-exercise fetal fraction ($p=0.38$). However, women with BMI <25 had an average fetal fraction change of -2.3% ($n=5$, $p=0.02$), while women with BMI ≥ 25 had an average change of $+0.5\%$ ($n=10$, $p=0.25$). Change in fetal fraction was not influenced by gestational age, maternal age, change in heart rate, or change in systolic blood pressure.

Discussion: Moderate maternal exercise may only impact fetal fraction when maternal BMI is <25. Further studies are required to confirm this association.

MEASURABLE RESIDUAL DISEASE (MRD) DETECTION BY NEXT GENERATION SEQUENCING (NGS) IN ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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Introduction: MRD, a key prognostic factor in ALL, is traditionally assessed by flow cytometry and/or allele specific oligonucleotide PCR (ASO-PCR). Here we describe validation and implementation of novel NGS technologies to measure MRD at clinically relevant time points.

Methods: Extracted DNA from bone marrow were analysed using the LymphoTrack® Diagnostic Assay Panel to detect IgH gene rearrangements. Sequence clonality was defined as $>2.5\%$ of total reads and $>2x$ frequency of the third most frequent sequence. A 100 cell equivalent spike-in control (LymphoQuant™) was utilised for normalisation.

Results: Results were concordant (78.18%) between assay methods with the exception of 12 samples. In 10 cases, MRD was detected by NGS at a lower level than flow cytometry and ASO-PCR, which were negative. MRD positivity by NGS corresponded with poor clinical outcomes in these patients. We demonstrate that the use of spike-in controls deliver clinically meaningful results.

Conclusion: MRD detection by NGS is complementary to testing using flow cytometry and ASO-PCR. NGS has the potential advantage of increased sensitivity, detection of clonal evolution and a rapid turnaround time. Normalisation of MRD levels to cell equivalents is required to suitably compare results with flow cytometry and ASO-PCR.

REVIEW OF 12 MONTHS OF COPY NUMBER VARIANT CALLING ON A CLINICAL NEXT GENERATION SEQUENCING PIPELINE

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Background: Next generation sequencing (NGS) provides the opportunity to detect not only single nucleotide variants and small indels, but also larger whole exon or whole gene deletions and duplications (CNVs).

Method: We implemented CNV calling in our NGS pipeline utilising the DECoN, XHMM and PanelCNV packages. We compared the performance with multiplex ligation-dependent probe amplification (MLPA) on our 1200 gene panel (R1kD) and with chromosomal microarray (CMA) on our whole-exome (WES) platform.

Results: On the R1kD, we reviewed 197 patient cases which had both NGS and MLPA. We detected 25 deletions and 7 duplications, all of which were detected by both methods. Seven other CNV calls from NGS were considered artefacts during IGV review due to the presence of pseudogenes and poor mapping. On the WES platform, we reviewed 37 patient cases which had both NGS and CMA and detected 11 deletions and 10 duplications by both methods. NGS called some additional smaller events which were not reported by CMA.

Conclusions: CNV analysis on NGS can be implemented in clinical pipelines. Based on review of the first 12 months of service we have moved some tests to NGS with MLPA only performed to check positive results.

HEPARIN INDUCED THROMBOCYTOPENIA IN PATIENTS WITH SEVERE RHABDOMYOLYSIS: A CASE SERIES

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Heparin-induced thrombocytopenia (HIT) is a known immunological complication of heparin administration which can cause life threatening thrombosis. Here, we report a series of three cases of HIT in the context of acute kidney injury due to rhabdomyolysis that presented to Launceston General Hospital, Tasmania, between December 2017 and December 2018. During this time, there were a total number of four patients with HIT, out of which three had rhabdomyolysis. Patients were all men with age between 29 and 68 years old. The causes of rhabdomyolysis were statin therapy (in two cases) and ischemia induced by compartment syndrome (in one case). All patients received unfractionated heparin and continuous renal replacement therapy for 2–11 days. 4Tscore was 4 in one patient and 5 in two other patients. None of patients had thrombosis complication. In all patients, anti-heparin/PF4 antibody was detected by BioRad Heparin/PF4 gel column assay and the diagnosis was confirmed by serotonin release assay. Patients received non-heparin anticoagulation. Platelet count recovered after cessation of heparin. Since rhabdomyolysis is an uncommon condition, the concomitant development of HIT in our 3 cases raises the suspicion whether administration of unfractionated heparin in patients with rhabdomyolysis poses an additional risk for developing HIT.

IDARUCIZUMAB RESISTANCE DUE TO A MARKEDLY ELEVATED DABIGATRAN CONCENTRATION – A CASE REPORT

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Dabigatran is a direct thrombin inhibitor licensed for treatment of venous thromboembolism and in stroke prevention for patients with non-valvular atrial fibrillation. It can be rapidly reversed when clinically indicated with idarucizumab, a humanised monoclonal antibody fragment.¹

A 64-year-old male taking dabigatran 110 mg daily for management of atrial fibrillation presented with abdominal pain and acute kidney injury. On presentation he was found to have a markedly elevated dabigatran concentration of 2230 ng/mL and deranged coagulation profile with a thrombin time of >200 seconds (RR 10–12 seconds) and INR of 11.2. Idarucizumab 5 g was administered and continuous renal replacement therapy commenced for persistent bleeding from a central line. However repeat dabigatran concentration performed 6 hours following idarucizumab administration had increased to 3722 ng/mL. A further dose of idarucizumab 5 g was administered 14 hours following the initial dose. Dabigatran concentration performed 4 hours following the second dose was 1787 ng/mL.

This case highlights a markedly elevated dabigatran concentration with significant coagulopathy in the context of kidney impairment and possibly concomitant amiodarone use. Despite idarucizumab the dabigatran concentration was markedly elevated, indicating idarucizumab resistance. Although uncommon, idarucizumab resistance must be excluded by repeat dabigatran concentration testing, particularly when the initial dabigatran concentration is elevated and/or kidney impairment.

Reference

1. Pollack CV Jr, Reilly PA, van Ryn J. Idarucizumab for dabigatran reversal—full cohort analysis. *N Engl J Med* 2017; 377: 431–41.

HYPEREOSINOPHILIA IN A PATIENT WITH REFRACTORY ECZEMA AND ALK-NEGATIVE ANAPLASTIC LARGE CELL LYMPHOMA

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A 41-year-old man with refractory eczema on immunosuppressants developed swinging fever and dyspnoea, with left pleural effusion and left axillary and groin lymphadenopathy (2–3 cm) found in physical examination. Clinically there was no eczema flare-up.

Laboratory tests showed hypereosinophilia ($79.1 \times 10^9/L$) and elevated LDH (520 U/L) and ALP (187 U/L). Some dysplastic eosinophils showing hyposegmented nuclei and hypogranular cytoplasm were noted on blood smears. Workup for parasites, allergy and autoimmune markers was unremarkable.

Thoracentesis yielded exudative effusate with numerous lymphocytes (WBC $>1000/mm^3$) but no malignant cell was evident. FNAC of lymph nodes found eosinophilic infiltrate only. Bone marrow examination revealed marked eosinophilia with similar dysplastic features and megakaryocytic hyperplasia, raising suspicion of myeloproliferative neoplasms. Karyotyping, molecular tests for JAK2 mutation and BCR-ABL1 fusion, and FISH for FIP1L1-PDGFR, PDGFRB and FGFR1 rearrangements were unremarkable.

Excisional biopsy of left groin lymph node was then pursued and demonstrated sheets of pleomorphic neoplastic cells with focal 'hallmark cells'. Anaplastic large cell lymphoma (ALK⁻ CD30⁺CD3⁺CD8⁺) was diagnosed. PET/CT scan revealed a stage-IV disease with extensive supra- and infra-diaphragmatic hypermetabolic lymph nodes and multiple bone involvements. The patient opted for brentuximab vedotin plus combination chemotherapy.

This case underscores the importance of exhaustive investigations and clinicopathological corroboration in the workup of hypereosinophilia.

NEUTRALISATION OF RIVAROXABAN INTERFERENCE ON APCR, FVIII AND FIX ASSAYS BY DOAC-STOP AND ANDEXANET ALFA

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Rivaroxaban is a widely utilised direct oral anticoagulant (DOAC), indicated for thrombosis treatment and prevention. Rivaroxaban binds to Factor Xa, affecting many haemostasis assays, including activated protein C resistance (APCR) and APTT based factor assays, including factor VIII (FVIII) and IX (FIX). Andexanet alfa is a clinical 'antidote' for anti-Xa agents, including rivaroxaban, neutralising activity *in vivo* and *in vitro*. DOAC-Stop is a commercial product that neutralises *in vitro* activity of DOACs.

We investigated whether rivaroxaban would induce false positive APCR and/or falsely reduced FVIII and FIX activity, and whether this could be neutralised by andexanet alfa or DOAC-Stop.

Four lyophilised plasma samples were prepared: normal plasma, and normal plasma spiked with rivaroxaban (200 ng/mL); rivaroxaban + DOAC-Stop; rivaroxaban + andexanet alfa. These were distributed to laboratories performing APCR and/or FVIII and FIX testing in RCPAQAP surveys. Data was analysed using robust statistics (mean, SD and CV).

In rivaroxaban 200 ng/mL samples, most APCR assays appeared largely unaffected, while FVIII and FIX assays showed a substantive reduction. Where assays were affected, DOAC-Stop neutralised rivaroxaban effects. Andexanet alfa did not completely neutralise the rivaroxaban effect, for APCR or FVIII and FIX, and unexpectedly lead to higher rates of false APCR than rivaroxaban alone.

THE CHANGING FACE OF TESTING FOR ACTIVATED PROTEIN C RESISTANCE (APCR) – A 10-YEAR RETROSPECTIVE

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Activated protein C resistance (APCR) is a hypercoagulable condition that increases the risk of venous thrombosis. The Royal College of Pathologists of Australasia Quality Assurance