Summary/conclusions: This study provides proof-of-principle that ddPCR reliably detects fetal HPA-1a signals from early gestation, allowing clinicians time to assess the need for IVIg management. This assay has the potential to provide a safer approach for managing such alloimmunised antenatal cases.

LIQUID BIOPSY FOR MANAGEMENT OF HAEMOLYTIC DISEASE OF THE FETUS AND NEWBORN

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Background: Haemolytic disease of the fetus and newborn arises from maternal allo-antibodies to red cell antigens, crossing the placenta. The most frequent antibodies after RhD, are Kell, Duffy and RhCE antigens.

Liquid biopsy in pregnancy management is concerned with the detection of cell-free fetal DNA (cffDNA) circulating in the maternal plasma. The accuracy for fetal RHD genotyping is well established.

Aim: To evaluate a maternal blood test to predict the fetal, non-RhD, blood group status.

Methods: A clinical collaborative study assessed the accuracy for a suite of assays to predict fetal Kell, Duffy, Rhc/C and Rhe/E status for women with respective antibodies ($n=90$, gestation age 8–37 weeks).

Droplet digital PCR technology, ddPCR, tested for single nucleotide variants (SNVs) associated with these blood group antigens. Infant cord blood serotypes were used as the primary outcome measure.

Results: Fetal specific signals were detected as early as 8 weeks gestation. Genotyping matched all available infant cord outcomes 45/45. Accuracy is 100% (95% confidence interval 91.96–100%).

Discussion: A clinical demand exists for liquid biopsy of maternal blood for fetal blood group genotyping. Droplet digital PCR is accurate and provides an evidence base to guide clinical care for at-risk pregnancies.

UTILISING GENEXPERT TO IDENTIFY CML IN THE PRIMARY CARE SETTING: TRIGGERS FOR TESTING AND PREDICTORS OF POSITIVE RESULTS

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The GeneXpert utilises quantitative PCR to detect BCR-ABL fusion transcripts in the molecular monitoring of patient with chronic myeloid leukaemia (CML).¹ Although GeneXpert hasn't currently been validated for the diagnosis of CML, BCR-ABL PCR on peripheral blood is frequently requested for this purpose. We performed a retrospective audit of BCR-ABL results in patients without established CML to assess test indications, triggers for ordering and the pre-test predictors of a positive BCR-ABL result. From January to November 2019, there were 178 tests with ten positive cases identified. All positive cases were initiated by a general practitioner triggered by a previous abnormal full blood count and film and had leucocytosis, neutrophilia and

myeloid left shift, with nine cases having a basophilia. The presence of basophilia and myeloid left shift amongst the 178 patients was highly predictive of an underlying myeloid neoplasm or clonal myeloid mutation on subsequent molecular testing (PPV 100% and 71%, respectively). This reinforces our laboratory protocol of contacting general practitioners when these features are identified on blood film to expedite further testing and referral. In order to reduce unnecessary ordering of BCR-ABL, education is required regarding the low yield of this test in the absence of leucocytosis or neutrophilia.

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METASTATIC GERM CELL TUMOUR PRESENTING AS ACUTE MEGAKARYOBLASTIC LEUKAEMIA AND DISSEMINATED MELANOMA: A CASE REPORT

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Since first recognised in 1985, studies have described the unique and rare association between haematological malignancies and germ cell tumours, most commonly acute megakaryoblastic leukemia (AMKL) and non-seminomatous germ cell tumours. A recent molecular study identified mutations in PTEN and TP53 in both tumours, supporting the theory that distinct genomic alterations underlie this rare association. It is also recognised that teratomatous components of mediastinal germ cell tumours have a tendency for secondary transformation to other malignancies including melanoma. We report a case of a 38-year-old man presenting with severe back pain. An MRI spine demonstrated diffuse marrow replacement and bony lesions concerning for metastatic disease. The patient had recently completed chemotherapy for a mediastinal germ cell tumour with teratomatous components on histopathology. A staging CT identified liver and splenic lesions suspicious for metastatic deposits. A peripheral blood film performed on admission was found to have circulating blasts with an immunophenotype consistent with megakaryoblasts. He subsequently underwent a bone marrow biopsy confirming AMKL with a TP53 mutation. A liver biopsy revealed necrotic tumour with immunohistochemistry consistent with metastatic melanoma. In summary, we describe a case of treated mediastinal germ cell tumour with secondary transformation to metastatic melanoma with synchronous AMKL.

UNKNOWN UNKNOWN: LIMITATIONS IN DETECTING TYPE II ANTITHROMBIN DEFICIENCY BY THE XA-BASED CHROMOGENIC METHOD

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Inherited deficiency of antithrombin (AT) occurs as a heterozygous quantitative reduction (type I) or qualitative reduction (type

II), and causes a high risk of thrombotic complications, including venous thromboembolism and obstetric morbidity. Recently updated international guidelines recommend incorporating a combination of a chromogenic thrombin or Xa-based functional assay, a progressive (heparin-independent, prolonged incubation) functional assay and a quantitative assay (e.g., latex agglutination or ELISA) in the evaluation of AT deficiency, however only chromogenic functional assays are routinely performed in Australian laboratories.¹ These functional assays have varying sensitivity to the wide variety of AT mutations, especially type II lesions, depending on the serine protease, incubation time and reagents used.^{2,3}

We describe four cases of suspected type II AT deficiency which demonstrated discrepant results between functional assays/reagents, and compare our historical cohort data of reduced AT activity before and after changing between commercial reagents. Trends in sensitivity of different assays/reagents reported in local and international quality assurance programs are also reviewed. These findings highlight the potential for under diagnosis of AT deficiency, the importance of fully characterising assay performance and understanding limitations in assay sensitivity – especially in these uncommon but clinically significant variants.

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BLOOD GROUP EXOME SEQUENCING DEFINES NULL AND WEAK KIDD (JK) PHENOTYPES: EVIDENCE FOR TRANSFUSION MANAGEMENT

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Background: The rare Jk(a-b-) Jk:-3 phenotype of the clinically significant Kidd blood group system (ISBT 009) is frequently encountered in the Polynesian population (0.272% compared to 0.002% in Caucasians).¹ Discrepancies between phenotype and genotype have revealed the limitations of conventional methods in differentiating true Jk(a-b-) phenotypes from those with variable antigen expression caused by genetic variants. We report two cases that were resolved by massively parallel sequencing (MPS).

Methods: The testing algorithm comprises red cell phenotyping plus genotyping using SNP array to determine Jk(a/b) antigen status. Sequencing using a targeted blood group exome panel on

the Illumina MiSeq is applied when discordant results are obtained.

Results: Case 1: Phenotype Jk(a-b-), genotype predicted Jk(a+b+). Sequencing indicated homozygosity for a silencing splice site variant, c.342-1G>A, predicting the 'null' Jk(a-b-) phenotype.

Case 2: Phenotype results were equivocal, suggesting either Jk(a+^wb-) or Jk(a-b-). Genotype results predicted Jk(a+b-). Sequencing indicated heterozygosity for nucleotide substitution, c.130G>A, defining the JK*01W.01 allele, consistent with weakened Jk(a) expression, therefore reported as phenotype Jk(a+^wb-).

Discussion: MPS defined blood group allelic variants to distinguish between null and weak antigen expression. This provides evidence to guide patient transfusion management and the precise provision of rare Jk(a-b-) blood inventory only when required.

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USE OF MOLECULAR MUTATIONS IN HIGH RISK MPN FOR ASSESSMENT OF BLASTIC TRANSFORMATION

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BCR-ABL negative myeloproliferative neoplasms are a heterogeneous group of clonal haematopoietic stem cell disease all of which have an intrinsic risk of evolution into acute myeloid leukaemia.¹ The frequency of leukaemia transformation varies according to the subtype with primary myelofibrosis estimated at 10–20% at 10 years followed by polycythemia vera at 2.3% in 10 years and essential thrombocytopenia varying widely from <1% to up to 10%.¹ Although blastic transformation often progress to acute myeloid leukaemia, transformation to acute megakaryoblastic leukaemia is rare in adult population being <1% of all AML.² We report a case of a 58-year-old lady who presented with progressive symptoms of fevers, back pain and fatigue with a 10 year history of polycythemia vera/essential thrombocytopenia overlap. Laboratory testing showed neutrophilia, mild anaemia with elevated lactate dehydrogenase and uric acid. Bone marrow biopsy showed marked infiltration of megakaryoblasts of various size and maturity with cytogenetics demonstrating hypertripody. Myeloid gene panel demonstrated mutations of JAK2, TP53 and TET2 which have been reported to concur high risk of blastic transformation in myeloproliferative neoplasms.³ In summary, we describe a case of PV/ET overlap with rare transformation into acute megakaryoblastic leukaemia.

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