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Irish External Quality Assessment Scheme CLG

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Blood Cell Morphology Review 2024
Dr Catherine Flynn, Consultant Haematologist, St James's Hospital

BCM: 150

DIAGNOSIS: Pyruvate Kinase Deficiency
This patient has PK Deficiency with partial splenectomy in 2003 and full splenectomy in 2005.

CLINICAL DETAILS: 24-year-old female. WCC = 12.4 x 10 9/L, Hb 7.8 g/dL, (Platelets = 524 x 10 9/L)

They had a significant reticulocytosis 42.2% (normal 0.4-1.8) with an absolute reticulocytosis of 992.2 x 10 9/L (normal 14.1 - 99.6 x 10 9/L).

BLOOD CELL MORPHOLOGY:
(Morphology comments: Prof Catherine Flynn, Consultant Haematologist).

My WCC Differential:
70% neutrophils
18% lymphocytes
3 % eosinophils
9% monocytes.
Rare NRBC seen, 1 per 100 WCC.

Blood film findings. Marked red cell changes with poikilocytosis and anisocytosis with spiculated (prickle cells) red cells, spherocytes, and polychromasia. Howell jolly bodies seen and rare NRBC. Thrombocytosis with some large platelets and minimal white cell leucocytosis with normal differential.

COMMENTS:
Most laboratories identified film findings consistent with haemolysis post-splenectomy and indeed this was the diagnosis. It wasn't unreasonable to consider a haemoglobinopathy given the significant red cell changes, although the features are not characteristic of classic haemoglobinopathies such as sickle cell or thalassaemia. The thrombocytosis, red cell changes and Howell jolly bodies are characteristic of the post-splenectomy state. Target cells, whilst seen, were not abundant. I would encourage all laboratories to make a call on a diagnosis. Well done to the 5 labs who had the confidence to call it and got the correct diagnosis.

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Other diagnostic tests beyond the film include indirect hyperbilirubinaemia, and positive Heinz body test. The PK enzyme levels can then be tested and nowadays, next generation sequencing can reveal mutations in the PKLR gene.
Pyruvate kinase (PK) deficiency is a rare, genetic, lifelong condition that affects red blood cells. It affects 3-8 per 1 million people. Symptoms vary greatly. How it affects one person can be significantly different from how it affects another person. In some instances, the disorder can be life-threatening at birth. Other individuals may have mild or no symptoms of the disorder and go undiagnosed into adulthood. The main symptom, haemolytic anaemia, can be associated with fatigue or low energy.

Everyone who has PK deficiency is born with it, even if they are diagnosed later in life. Pyruvate kinase deficiency is an autosomal recessive condition caused by mutations in the PKLR gene. It is the most common glycolytic defect causing congenital non-spherocytic haemolytic anaemia. The pyruvate kinase enzyme is involved in a critical energy-producing process in glycolysis. PKLR gene mutations result in reduced pyruvate kinase enzyme function, causing a shortage of ATP in red blood cells and increased levels of other molecules produced earlier in the glycolysis process. The abnormal red blood cells are entrapped by the spleen and destroyed, causing haemolytic anaemia and an enlarged spleen. It is an important diagnostic consideration in neonates with presentations outside of the classic indirect hyperbilirubinaemia, including in newborns with skin extramedullary haematopoiesis and hypoxemia.

A recent international observational registry study has collected data on these patients with PK deficiency to try to understand the clinical spectrum of the disease. Perinatal complications are common, including anaemia that require transfusions, hyperbilirubinaemia, hydrops, and prematurity. Nearly all newborns required treatment with phototherapy, and many required treatment with exchange transfusions. Children aged 5 years and younger, were often transfused until splenectomy. Splenectomy was associated with a medium increase in haemoglobin and a decreased transfusion burden in patients. Predictors of a response to splenectomy included higher pre-splenectomy haemoglobin, lower indirect bilirubin and missense PKLR mutations. Post-splenectomy thrombosis has been reported in a small number of patients. The most frequent complications of PK deficiency include iron overload and gallstones, but other complications such as aplastic crises, osteopenia/bone fragility, extramedullary haematopoiesis, post-splenectomy sepsis, pulmonary hypertension, and leg ulcers are not

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uncommon. Overall, approximately 30% of patients require a splenectomy and cholecystectomy. In those who had a splenectomy without simultaneous cholecystectomy, 48% later required a cholecystectomy. Although the risk of complications increases with severity of anaemia and a genotype-phenotype relationship was observed, complications were common in all patients with PK deficiency.

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BCM151:

DIAGNOSIS: ICC Provisional entity: B-ALL, ETV6:RUNX1-like

DIAGNOSIS: (WHO HAEM5) B-lymphoblastic leukaemia/lymphoma with ETV6 deletion.

CLINICAL DETAILS: 6-year-old female. WCC = 25.2 x 10 9/L, Hb = 8.8 g/dL.

BLOOD CELL MORPHOLOGY
(Morphology comments: Prof Catherine Flynn, Consultant Haematologist).

My Differential
85% blasts, 6 neutrophils, 3 bands, and 6 lymphs, 1 NRBC

Blood film findings:
Leucoerythroblastic blood film with a prominent monomorphic immature lymphocytic population. Marked thrombocytopenia.

Additional Test Results:
Neutrophils 3.28, Lymphocytes 2.27, Platelets 26.
LDH 3827, CRP <5, normal electrolytes.
B lymphoblastic ALL (Immunophenotyping PB CD45w/CD19+/CD10+, TDT+/cytoCD3-MPO-/CD79+
Immunophenotyping on BM: CD45+w/CD19+/CD10+, TDT+/cytoCD3-MPO-/CD79+/CD20-, cCD3-
CSF no blasts seen, however flow cytometry positive for blasts with similar immunophenotype (CD20-)
Cytogenetics Microarray- deletion ETV6 (12p13.2)
B-ALL with CNS1
Day 15- BM- MRD day 15- MRD detected, no morphological evidence of leukaemia in BM

COMMENT:
Almost every centre correctly identified acute leukaemia and most agreed B-ALL was the most likely. 3 labs did not offer a diagnosis, which is disappointing although their differential suggested acute leukaemia with a blast %>20%.
The rational for continued films like this for IEQAS review is to quickly identify a leukaemia diagnosis and move it forward for definite testing including immunophenotyping, cytogenetics and molecular analysis. It is critical that morphology skills are kept attuned

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to this possibility, because to date the advanced analysis needed to make the definite diagnosis are not typically available on call and the actions and decisions made on this young patient will be critically determined by a call from the lab to say that this is leukaemia.

Additional Comment

Deletions: ETV6 is frequently deleted in haematological malignancies. The deletion of the normal (untranslocated) ETV6 allele in the presence of a translocation affecting ETV6 is quite frequent, notably in patients with ETV6-RUNX1, ETV6-NTRK3, ETV6-ABL1, ETV6-ACSL6 and ETV6-STL fusion. Deletion of an ETV6 allele has also been observed in the absence of rearrangement of the second allele.

Some recent data suggests that B-ALL associated with ETV6 may have a genetic predisposition and further testing on family members may require testing to counsel in the potential indications especially if a family sibling donor is being considered as a transplant donor. A preceding thrombocytopenia can occur before definite transformation to ALL but may children will never had had a blood test previously.

In B-ALL- gene deletions occur in about 60% of cases

On review of the literature, it is not clear that the ETV6 deletion alone has prognostic value however in this case after 15 days into the first course of treatment the disease was still detectable by (minimal residual disease MRD) testing on the bone marrow of this girl and this is a poor prognostic factor.

New methods of classification, minimal residual disease testing and monitoring with the incorporation of molecular testing are attempts by scientist and clinicians to try and better plan treatment including addition of targeted treatments and consideration of the place and timing for high-risk procedures such as an allograft.

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BCM152:

DIAGNOSIS: Homozygous Sickle Cell Disease and PE post C-section in 2014

CLINICAL DETAILS: 36 year old female. WCC = 11.77 x 10 9/L, Hb = 8.0 g/dL.

BLOOD CELL MORPHOLOGY
(Morphology comments: Prof Catherine Flynn, Consultant Haematologist).

FBC results: Hb 8.0, WCC 11.77, platelets 399, MCV 88.8, reticulocytes 281.9 (35-123)
Bands of HbS (74.7%), HbF(19.0%) and HbA2 were found on capillary electrophoresis

Blood film findings:
My Differential: 65% neutrophils, 30% lymphocytes, 2 monocytes, 3 eosinophils (100 cells), 5 per 100 NRBC

Film shows marked red cell anisocytosis with NRBCs, sickle cells, target cells and macrocytes with multiple Howell-Jolly bodies. Large platelets and left shifted myeloid series.

Excellent performance by most labs with correct identification of the blood film findings and a consistent diagnosis. 3 labs did not offer a diagnostic comment and we continue to try and emphasise the importance of this. Morphological findings were correctly identified in the majority.

A lot of labs mentioned the possibility of a combined HbS/C and this was likely due to the abundance of target cells, however as mentioned below target cells are not diagnostic in themselves.

COMMENT:
Sickle cell disease (SCD) is a haemoglobinopathy characterized by vaso-occlusive episodes and haemolysis. These crises are more critical during pregnancy and any mother with sickle cell disease should be looked after within a specialised team to ensure optimal care and attention to mother and foetus.

Haemoglobin S is prone to polymerize at low oxygen tension, causing the red cell to become sickle shaped, more rigid and sticky. Evaluation of blood cell morphology and

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blood counts and red cell genotype are important components of the patient evaluation and can be the first time sickle cell disease is diagnosed, as sometimes there is a cultural reluctance to admit this diagnosis.

The diagnosis is usually accomplished by evaluation of the blood film, performing a full blood count (FBC), and the use of haemoglobin electrophoresis. A typical blood film from an SCD patient shows anisocytosis, poikilocytosis, polychromasia, nucleated erythrocytes, sickled cells, and irregular contracted cells. All these abnormalities are present in BCM152. Once sickle cell disease is diagnosed, referral to a haematologist is important and red cell genotyping should be considered if transfusion is considered necessary.

The appearance of target red cells raises the possibility of other haemoglobinopathies (such as haemoglobin S, haemoglobin C, and haemoglobin E), as well as acquired disorders such as liver disease, iron deficiency and post splenectomy.

The presence of Howell-Jolly bodies is associated with splenic dysfunction, which progressively appears during aging or after splenectomy in patients with sickle cell disease.

Additional Comment:
Sickle cell disease (SCD) consists of a group of haemoglobinopathies in which individuals inherit haemoglobin variants derived from single point mutations, that causes morphological abnormalities in the red blood cells (RBC). Sickle cell anaemia (SCA) is characterized by the homozygosity for haemoglobin S (HbS) and is the most frequent and severe form of the disease. The point mutation of GAG to GTG in the sixth codon of the β (beta) globin gene (HBB), which replaces the glutamic acid for a valine, leading to HbS formation. HbS forms long polymers when the oxygen tension is low, due to the hydrophobic interaction of valine (at 85 position in the globin chain) and phenylalanine (at 88 position in the globin chain). Red blood cells of SCA individuals are less flexible since the polymers lead to rheological and biochemical changes and hence they impair the blood flow causing vaso-occlusion (VO).

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BCM153:

DIAGNOSIS: WHO/ICC Diagnosis

By Morphology: Polycythaemia Vera in transformation to AML

Following review of marrow with cytogenetic and molecular results:

ICC: AML with myelodysplasia-related cytogenetic abnormalities

WHO: Acute myeloid leukaemia- myelodysplasia related

CLINICAL DETAILS: 76-year-old male. WCC = 1.2 x 10 9/L, Hb = 10.5 g/dL.

The patient has a background of Polycythaemia Vera (PV) and he presented with pancytopenia.

BLOOD CELL MORPHOLOGY

(Morphology comments: Prof Catherine Flynn, Consultant Haematologist).

My differential:

20% neutrophils, 65% lymphocytes, 2 monocytes, 6 eosinophils, 5% blasts and 2 Myelocyte. No NRBCs were seen.

Film red cell macrocytes, large platelets and 5% circulating blasts.

Additional results:

Marrow report: particulate hypercellular marrow, with decreased megakaryocytes, the majority of which have hyper-lobated nuclei in keeping with known MPN, myeloid series present to maturation with occasional hyper segmented neutrophil, erythroid series showed mild to moderate dyserythropoiesis. Blasts in marrow 17%.

Immunophenotype on BM blasts: CD34, CD117+, CD38+, CD68+, HLADR+, cytoMPO+

A pathogenic variant in the jak2 gene that is associated with myeloproliferative neoplasms (MPN) was detected in this sample.

Variants classed as pathogenic were detected in the IDH1, JAK2, MPL, Runx1 and SRSF2 genes.

Mutations in Idh1, Runx1 and SRSf2 are considered as adverse high molecular risk mutations in patients diagnosed with PV according to NCCN.

Cytogenetics: 46XY -7[5] and 46XY[15].

COMMENT:

All labs noted a small number of blasts (1-10%) and many identified red cell macrocytes and large platelets. Some considered the lymphoid cells abnormal and of course a clinical

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history would have assisted with this interpretation; some went as far as going down the route of a lymphoproliferative disorder; prompt clinical screening by a good history and immunophenotyping would have assisted with this.

The blasts are large and while no Auer rods were seen, I believe the blasts were large enough to warrant a myeloid blast screening prior to a lymphoproliferative screen.

2 laboratories offered no diagnostic comment; this is an ongoing problem which we urge participants of this quality program to rectify. It is worth the effort to try and determine a diagnosis even if incorrect. The scheme emphasis is on morphological skills not the diagnostic comment, but it is good to force ourselves to make an educated diagnostic comment.

Additional Comment:

Among myeloproliferative neoplasms, polycythaemia vera (PV) is associated with a chronic disease course. Leukaemic evolution occurs rarely but has a grim prognosis. The interval between diagnosis and leukemic evolution is highly variable, from a few years to >20 years. Continued lifelong monitoring is recommended whether on or off cytoreduction treatment, to monitor for disease transformation. There is a lot of interest that new molecular tests may assist in better predicting who and when patients will transform. However, the dilemma continues as to how (peripheral blood or marrow) and how often, one should test these patients with a chronic illness. Many patients live with these chronic myeloproliferative illnesses and live normal lives and frequent marrow monitoring would likely be considered unacceptable intervention by most.

Recent study: (https://doi.org/10.1182/bloodadvances.2020002271).

There is a continued dilemma whether patients with a new diagnosis of polycythaemia should have molecular testing, and what to advise patients with this information, since the field is still in evolution. This remains an active area for discussion and research, but current guidelines recommend risk stratification on age and thrombosis history.

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BCM154:

DIAGNOSIS: Refractory AIHA and JAK2 positive MPN

CIINICAL DETAILS: 76-year-old Male, WCC = 9.5 x 10 9/L, Hb = 7.8 g/dL (Plt = 322 x10 9/L)

BLOOD CELL MORPHOLOGY:

(Morphology comments: Prof Catherine Flynn, Consultant Haematologist).

My differential: 79% Neutrophils, 13% Lymphocytes, 8% Monocytes, 4 NRBC/HPF.

Overall, labs identified the correct morphological findings of spherocytes, Howell Jolly bodies and dysplastic neutrophils. Most made a good attempt at the diagnosis which varied from myelodysplasia to autoimmune haemolytic anaemia. The precise diagnosis in this case was difficult, as it was a combination of diagnoses on complex therapy, which was likely contributary. I was relieved to see that many were not convinced by basophilic stippling, but some did comment on punctate basophilia which is a fair comment.

Additional results:

Complete FBC:

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Name Test	Result	Ref.Range	Ms.Unit(s)
WCC	9.5	4.0-11.0	10 9/L
NEUT	NA		10 9/L
LYMP	NA		10 9/L
MONO	NA		10 9/L
EOSIN	NA		10 9/L
BASO	NA		10 9/L
RCC	1.89	4.60-5.70	10 12/L
HB	7.8	13.5-18.0	g/dL
HCT	0.226	0.430-0.510	Ratio
MCV	119.6	83.0-99.0	fL
MCH	41.3	26.7-32.5	pg
MCHC	34.5	30.8-34.6	g/dL
RDW	21.2	11.0-15.0	
PLAT	322	140-450	10 9/L
NRBC	0.3		10 9/L

Test Name	Result	Ref Range	Units	Comment
WCC	9.5	4.0-11.0	10 9/L	
RCC	1.89	4.60-5.70	10 12/L	Low
Hb	7.8	13.5-18.0	g/dL	Low
HCT	0.226	0.430-0.510	Ratio	Low
MCV	119.6	83-99	fL	High
MCH	41.3	26.7-32.5	pg	High

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MCHC	34.5	30.8-34.6	g/dL	
RDW	21.2	11.0-15.0		High
PLTs	322	140-450		
NRBC	0.3		10 9/L	

Reticulocytes: 12%, absolute reticulocytes: 226.8 x10 9/L (NR 14.1-99.6)
LDH: 391 U/L (NR 135-250)
Abnormal Film with marked red cell changes, NRBC 4 per 100 cells. Multiple Howell Jolly bodies, pappenheimer bodies, spherocytes and macrocytes were present. There were large platelets, and left shifted myeloid cells with dysplastic changes and hypo granular forms. On my extended review of the film, I was could not find much basophilic stippling but accept that it may have been present.

COMMENT:

This man was diagnosed with Autoimmune haemolytic anaemia in Oct 2021 and received multiple treatments including prednisolone, rituximab, mycophenolate mofetil, immunoglobulin and later azathioprine with multiple relapses.
Simultaneously following a significant rise in WCC and Plt count on erythropoietin and hydroxycarbamide, he was also diagnosed with a JAK2+ Myeloproliferative disorder in February 2023. His spleen was enlarged but intact.

Gene	c.HGVS	p.HGVS	VAF	Variant reads*	Classification
ASXL1	c.1900_1922del	p.Glu635Argfs*15	45%	748	Likely Pathogenic
JAK2	c.1849>T	p.Val617Phe	8.3%	130	Pathogenic
TET2	c.3250C>T	p.Gln1084Ter	47%	915	Likely Pathogenic

On further testing using myeloid NGS on a bone marrow sample, this patient has 3 myeloid mutations, as outlined above.
ADDITIONAL COMMENT:
ASXL1 mutations are considered high risk molecular markers and are associated with poorer prognosis in myeloproliferative disorders, according to the NCCN MPN guidelines (Version 3.2022). These guidelines acknowledge that mutations in TET2 have been reported in MPNs, however, mutations within the TET2 gene currently have limited prognostic significance.
A reminder of the definition of a Howell-Jolly body: a small, round, dark purple inclusion within the red blood cell. Howell-Jolly bodies are nuclear remnants that occur where there is no spleen, or a non-functioning spleen known as asplenia (Wright-Giemsa stain).

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Pappenheimer bodies (from ASH image bank)* are located peripherally in the erythrocyte: abnormal basophilic granules of iron found inside red blood cells on routine blood stain (Wright-Giemsa stain).

View Papperheimer bodies image* here:
https://www.ieqas.ie/resources/pdf/BCM%20Report%20images/BCM154%20-%20Pappenheimer%20Bodies%20Image.pdf

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BCM 155:

DIAGNOSIS:

CLINICAL DETAILS: 77-year-old female. WCC = 6.1 x 10 9/L, Hb = 7.5 g/dL (Plt 61 x 10 9/L)

NOTES: