IEQAS
Irish External Quality Assessment Scheme CLG

B06 Nutgrove Enterprise Park, Rathfarnham, Dublin 14
www.ieqas.ie info@ieqas.ie P: 01 4957356

Blood Cell Morphology Review 2023
Dr Catherine Flynn, Consultant Haematologist, St James's Hospital

BCM: 144

DIAGNOSIS: Homozygous Sickle cell disease in an adult female with a co-existent vaso-occlusive crisis.

CLINICAL DETAILS: 26 year old female. WCC = 20.5 x 10 9/L, Hb = 8.1 g/dL, Plt = 271 x 10 9/L

ADDITIONAL COMMENT: The film shows significant red cell changes with macrocytes, hypochromia, tear drops and target cells. There are prominent boat shaped and sickle shaped cells. There are Howell Jolly bodies in some of the red cells, consistent with an auto splenectomy
seen in many adult patients with sickle cell disease. The film also shows a leucocytosis comprising both neutrophils and lymphocytes, with some monocytes and eosinophils, consistent with a reaction to infection.

My Differential:
44% neutrophils
45% lymphocytes
9% monocytes
2% eosinophils

This was almost universal in the correct diagnosis. All laboratories correctly identified sickle cells and most diagnosed Sickle cell disease with
a lymphocytosis. Some laboratories thought there might be coexistent haemoglobinpathy disorders, such as Hb C. There were insufficient
target cells to support this. There are visible Howell Jolly bodies, and these are consistent with an auto splenectomy in an adult sickle cell
patient (the patient has not had a surgical splenectomy). Howell-Jolly bodies are fragments of DNA and are typical in the peripheral smears
of individuals with sickle cell disease following auto-splenectomy. Howell Jolly bodies are a single round dense structure with a regular border
in contrast to Pappenheimer bodies which appear as single or multiple irregular inclusions/granules within the red cell. Pappenheimer bodies
are abnormal granules of iron found inside red blood cells. Both Howell Jolly bodies and Pappenheimer bodies are seen in this film and were variably identified by some laboratories.

With regard to the leucocytosis, this was considered reactive in most laboratories, as is the case. Others thought it might reflect an
underlying lymphoproliferative condition which is possible, but unlikely. Some laboratories identified rare blasts and NRBCs; these findings
are a reflection of the reactive process ongoing in this young patient.

Background:
This film is of a 26 year old female, originally from Nigeria who moved to Ireland recently. She has a background of homozygous sickle disease and reportedly has had admissions with cardiac failure previously. On the day of this film, she presented with a vaso-occlusive crisis to her local A&E department, which developed into an acute chest syndrome. She has an HbA level of 12.1%, an elevated HbF level at 6.5% (Ref range: 0-2%), an Hb A2 level of 3.5% and Hb S level of 77.2%. The elevated Hb F level is likely

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due to ongoing treatment with hydroxycarbamide. She had a reticulocyte count of 17% (Ref range: 0.4-1.8) and an absolute reticulocyte count of 391 x 10 9/L pf 391- elevated (Ref range: 14-99) and had a bilirubin of 44 µmol/L (Ref range: 0-21) consistent with ongoing haemolysis due to her sickle cell
disease and associated vaso-occlusive crisis. No specific bacteria was identified and the precipitant of the crisis was not known although the
patient thinks the cold temperatures were contributory.

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BCM 145

DIAGNOSIS: Transient abnormal haematopoiesis with trisomy 21

CLINICAL DETAILS: 2 day old male. WCC = 16.4 x 10 9/L, Hb = 14.9 g/dL, Plt 28 x 10 9/L

My Differential: 19% blasts, 10% lymphs, 7% monocytes, 1% basophils, 26% metamyelocytes and 36% neutrophils, with 32 NRBCs in same field.

Film Review: Leucoerythroblastic film with dysplastic hypogranular neutrophils and left shift. Multiple NRBCs with target cells, macrocytes and polychromasia. Severe thrombocytopenia also noted.

ADDITIONAL COMMENT:
Most laboratories identified a leucoerythroblastic film with varying numbers of blasts (4-27%). I was surprised to note 3 laboratories did not report blasts in their differential, which is concerning. At least 2 laboratories mentioned NAIT which is a differential to include in a term infant with unexpected thrombocytopenia, however, the film would show an isolated thrombocytopenia with no WCC or red cell findings, and therefore in this case, it is not a valid conclusion. The WCC changes including left shift and multiple NRBCs would not be seen in NAIT.

The blasts are more heterogeneous, with immature monocytes also seen, which may have influenced the differential. Infant leukaemia is a reasonable conclusion. Sepsis should be excluded and indeed without clinical details, this would not be possible, although I think sepsis alone could not account for an increase in the blast numbers >5% in PB. The number of NRBCs is striking and worthy of comment. Several labs mentioned possible prematurity as contributory, and most premature neonates have excess NRBCs in PB without any other pathology, often detected in 'growing bloods' and severe sepsis in a premature infant could be considered.

TAM should always be considered in a film such as this, and it is a timely reminder to those of us who examine neonatal films with sparse clinical data. In the literature, blasts in TAM can have features of megakaryoblasts with cytoplasmic blebbing. These features were not apparent to me in this film, but this blood film should have been concerning to any scientist to warrant medical review and immunophenotyping.

Transient abnormal myelopoiesis (TAM) is a myeloid proliferative condition with leukaemic potential, almost exclusively seen in infants with trisomy 21 (Down syndrome [DS]).

Neonates with Down syndrome (DS) have a marked propensity to develop the unique myeloproliferative disorder referred to as transient abnormal myelopoiesis (TAM), transient myeloproliferative disorder, or transient leukaemia. In the majority of cases, TAM resolves spontaneously in ≤3 months, but approximately 10% of patients with TAM die from hepatic or multi-organ failure. Recent studies have identified risk factors for infant death due to TAM and indicate the efficacy of low-dose cytarabine for treatment of severe manifestations of TAM.

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Patients with TAM present with various haematological abnormalities. Leucocytosis and thrombocytopenia are common, and white blood cell (WBC) count may exceed 100 × 109/L. Increased neutrophils, myelocytes, monocytes, and basophils are frequently observed in peripheral blood smears, and, interestingly, there are cases of TAM involving prominent eosinophilia. TAM is characterized by a varying degree of increased blast cells in the peripheral blood. Transient abnormal myelopoiesis is a unique myeloproliferative disorder in newborns with DS, who present with circulating blasts that are morphologically and phenotypically similar to AML. The blasts have morphologic and immunologic features of the megakaryocytic lineage, but the morphology of TAM blasts is variable in practice. Clinical diagnosis of TAM is based on the presence of characteristic blasts in peripheral blood smears. Given that TAM blasts carry mutations of GATA1 in addition to trisomy 21, positivity for GATA1 mutation on NGS confirms the diagnosis of TAM, but the percentage of TAM cells varies enormously, both temporally and between patients, and there are no clear diagnostic criteria for TAM.

The immunophenotype of TAM blasts is the same as that of AMKL, with blast cells positive for stem cell markers (CD34, CD117), myeloid markers (CD13, CD33), platelet glycoproteins (CD36, CD42, CD61), CD56, and CD7, with variation among cases.

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BCM 146

DIAGNOSIS: Acute Monocytic Leukaemia.
Refined diagnosis based on cytogenetic and molecular data:
AML with Recurring Genetic Abnormality (NPM1) - WHO Haem 5
AML with mutated NPM1 ≥ 10% - ICC Classification

CLINICAL DETAILS: 62-year-old male. WCC = 81.6 x 10 9/L, Hb = 10.5 g/dL, PLT 85 x 10 9/L.

BLOOD FILM MORPHOLOGY (Please note these comments are based on full blood count and blood film morphology alone).
Morphology comments: Prof Catherine Flynn, Consultant Haematologist.

Differential: 10% neutrophils, 1% myelocyte, 24% lymphocytes, 65% immature monocytes (23% monoblasts, 42% monocytic precursors).

Comment: Leucoerythroblastic film with a large proportion of monocytic precursors including monocytic blasts, scant myeloid precursors.
Note the classification of AML proves difficulty with this case and for one of the first occasions, I believe the morphology has become redundant except for the blast percentage (clearly >10%).

Further Investigations: Cytogenetics and molecular genetics
Karyotype: 46XY + 2 [7], 46XY [13]
Flt3-ITD negative
NPM1 exon 12 mutation positive
NGS: BRAF, IDH2, NPM1, SRSF2 mutations present

ADDITIONAL COMMENT:
All laboratories found AML and most expressed concern about monoblastic/monocytic lineages which is reassuring. Three labs did not make a diagnostic comment and I would encourage everyone to make an educated estimate of diagnosis. The differentials were very uniform with a small number of myeloid and lymphoid cells and a majority of early monoblastic and monocytic precursors. This case highlights the difficulties with the new classifications of AML, which should be used to reflect the prognosis and to in treatment planning including eligibility for clinical trials.
NPM1 mutations represent the most common genetic lesion in adult acute myeloid leukaemia (AML; about one third of cases), and they act deterministically to cause the aberrant cytoplasmic delocalization of NPM1 mutants. BM samples are typically hypercellular and most NPM1+ AML are myelomonocytic (FABM4) and monocytic (FAB M5) but all FAB categories are represented. About 23% of cases may demonstrate some multilineage dysplasia, not very evident in this case.

NPM1 mutations frequently co-occur with mutations of FLT3, DNMT3A, and IDH1/2 genes. Prognosis may vary according to the associated mutations.
Currently there is an active clinical trial in Ireland for patient with IDH1/2 mutations (Hovon 150) and participation in clinical trials should be encouraged as it allows access for patients to drugs such as IDH1 and 2 inhibitors not otherwise available.

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A limitation to the wide application of genomic classification, is that NPM1-mutated AML is fragmented into many small molecular subsets whose prognostic impact may be difficult to study in clinical trials. Measurable residual disease (MRD) assessment in NPM1-mutated AML patients may help to overcome this limitation. NPM1 mutations are ideal targets for MRD monitoring because they are frequent, stable at relapse, and not found in subjects with clonal haematopoiesis.

Appendix:
(Links checked by IEQAS 17/05/2023)
The 5th edition of the WHO Classification of Haemtolypmhoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia (2022) https://doi.org/10.1038/s41375-022-01613-1)

International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood (2022) https://doi.org/10.1182/blood.2022015850

Diagnosis and Management of AML in Adults: 2022 recommendations from an international expert panel on behalf of the ELN.
European Leukaemia Net ELN Blood (2022) https://doi.org/10.1182/blood.2022016867)

NOTES:

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BCM 147

DIAGNOSIS: Hairy Cell Leukaemia (WHO Haem 5th edition).
HCL (classic - WHO 2008).
Hairy Cell Leukaemia is a mature B-cell neoplasm with distinctive clinicopathologic features and BRAF p.V600E (NP_004324.2) somatic mutation in ≥95% of cases. Other splenic small B-cell lymphomas usually lack BRAF mutations.

CLINICAL DETAILS: 39 year old female. WCC =3.5 x 10 9/L, Hb 8.3 g/dL, Platelets = 33 x 10 9/L
Monocyte count at diagnosis 0.9 (0.2-0.8)
Neutrophil count at diagnosis 0.3 (2.0-7.5)

BLOOD CELL MORPHOLOGY:
Morphology comments: Prof Catherine Flynn, Consultant Haematologist.
This film demonstrates a prominent lymphocytosis with a slightly elevated monocyte count. 73% of lymphocytes were small with clumped chromatin and 26% were larger with folded nuclei, prominent nucleoli and hairy cytoplasmic projections.

I was underwhelmed by the red cell anisocytosis/hypochromia, and on review, the MCV on the day of diagnosis was elevated at 106.6.
She had a neutropenia at diagnosis (0.3), with a red cell macrocytosis 106.6 (83-98) and B12, folate and ferritin were all normal. Her reticulocytes were 2.3% (absolute 55.4); low for the severity of the anaemia.

My differential on review of the film:
86% lymphocytes
1% eosinophils
12% neutrophils
1 % monocytes

Almost all laboratories got the correct diagnosis of Hairy Cell Leukaemia, some were less willing to commit and reported a lymphoproliferative condition with monocytopenia and lymphocytosis.
Three labs did not make a definite diagnostic comment and I believe it is wise to try to make a diagnosis in all cases, even if not convinced. They all mentioned hairy cells in their differential.

COMMENT:
Hairy Cell Leukaemia is an uncommon haematologic malignancy characterized by pancytopenia and marked susceptibility to infection.
Classically, this diagnosis is an incidental finding on a routine FBC, and indeed this patient had bloods sent in from GP when having her thyroid function tests checked and it was incidentally noted to show a pancytopenia with hairy cells on the PB film. Further questioning on presentation revealed bruising for some time and heavy menstruation.
This is a reminder that close inspection of a blood film in pancytopenia is needed and if haematinics and TFTS are non-diagnostic, a marrow examination should be considered soon.

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Hairy Cell Leukaemia accounts for less than 2% of all leukaemias. Its incidence is 0.3 cases per 100,000 individuals, with an average male-to-female ratio of 1.5 - 2:1, and a median age at diagnosis of 58 years. The incidence is approximately three times higher in White than in Black populations. This person was of White Caucasian ethnicity.

PB immunophenotyping can clinch the diagnosis in these cases. Identification of $BRAF^{V600E}$ mutation by allele-specific polymerase chain reaction, sequence analysis is also recommended by the newer classifications.

Tremendous progress in the management of patients with this disease has resulted in high response rates and improved survival, yet relapse and an appropriate approach to re-treatment present continuing areas for research.

Within the differential diagnosis, it is reasonable to consider: Hairy Cell Leukaemia, Hairy Cell Leukaemia variant; Splenic Marginal Zone Lymphoma; Splenic Diffuse Red Pulp Small B-cell Lymphoma, although the immunophenotypic profiles will help confirm the diagnosis.

The immunophenotypic profile of the leukaemic cells is critical for establishing this diagnosis. Immunophenotypic characterization of the peripheral blood mononuclear cells reveals light chain restriction of either κ− or λ-expressing populations of B cells. The characteristic immunophenotype of CD19+, CD20+, CD11c+, CD25+, CD103+, CD123+ co-expressing cells confirms the diagnostic features of HCLc. These cells are intensely stained for CD200 expression but negatively stained for CD27 antigen. In contrast, leukaemic cells in patients with HCLv, are most often negative for CD25 and CD123, and most of these patients will not be monocytopenic.

In 2008, the World Health Organization determined that the classic form of Hairy Cell Leukaemia (HCLc) should be recognized as separate from the rarer variant of this disease, called Hairy Cell Leukaemia variant (HCLv). The observation that a specific mutation, $BRAF^{V600E}$, is present in the overwhelming majority of patients with HCLc and absent in HCLv, validates this and concurs with the clinical observation that HCLv follows a different clinical course and response to therapy.

Demonstration of the $BRAF^{V600E}$ mutation could also be important for those who do not respond to standard therapy or have multiple relapses. Inhibitors of $BRAF^{V600E}$ have provided responses in patients who are resistant to standard therapy. Consequently, it is now recommended that all patients with HCL be evaluated for this mutation by using a sensitive molecular assay that can detect the often few (<10%) leukaemic cells present in the peripheral blood or in bone marrow aspirates diluted with blood as a result of dry tap.

ADDITIONAL COMMENT:
This young lady received 5 days of sub-cutaneous Cladribine in October 2022, with consolidation of 4 weekly doses of Rituximab-a CD20 antibody and now has a normal PB FBC.
Subsequent marrow examination reveals Hairy Cell Leukaemia in remission although a small clone was still detectable by flow cytometry indicating a low risk of recurrence. No further treatment is planned.

The most frequent cause of death among patients with HCL is infection. Because these patients often present with pre-existing neutropenia and/or monocytopenia, bacterial,

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viral, and opportunistic infections should be anticipated. In addition, the primary therapy for HCL is immunosuppressive, and patients may be placed at further risk for infection during treatment. Purine analogs confer prolonged suppression of immune effector cells (eg, CD4+ T cells) and induce profound and prolonged neutropenia.

Because patients with HCL who have previously been treated with purine analogs have profound and persistent lymphopenia, they should probably receive irradiated blood products, if a transfusion is indicated, to prevent transfusion-associated graft-versus-host disease. Furthermore, hepatitis history should be documented with consideration for suppressive anti-viral treatment of those who are positive for hepatitis B surface antigen. Patients can have severe liver toxicity after immunosuppressive therapy, if reactivation of viral hepatitis should occur. Therefore, screening for previous exposure to hepatitis before therapy for the disease is highly recommended.

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BCM 148

DIAGNOSIS: Acquired Thrombotic thrombocytopenia purpura (TTP).

CLINICAL DETAILS: 61 year old male. WCC = 8.5 x 10 9/L, Hb = 8.2 g/dL. Plt 17 x 10 9/L.

BLOOD CELL MORPHOLOGY:
(Morphology comments: Prof Catherine Flynn, Consultant Haematologist).
This film shows striking red cell changes with marked anisopoikilocytosis , polychromasia and numerous schistocytes, varying from 4-5/HPF to other areas where there were 20/HPF. The schistocytes were classic with pointed edges, and nucleated red cells were seen but were not high in number.
There was also a marked thrombocytopenia.
There is a marked left shift in the WCCs with occasional blasts, but insufficient to diagnose a co-existent leukaemia. There were some reactive lymphoid cells suggestive of an inflammatory process, but I was not convinced of an underlying lymphoproliferative condition.

My Differential:
56 Neutrophils, 11 Metamyelocytes, 3 Myelocytes, 24 Lymphocytes and 6 Monocytes (100WCC) with a single NRBC.

COMMENT:
There was a good performance by all laboratories, who correctly identified the morphologic findings including schistocytes and thrombocytopenia, and the majority saw spherocytes and polychromasia. These findings in the peripheral blood with > 5 schistocytes per HPF were clear, and it is the responsibility of scientists to alert clinical staff asap when this film is discovered day or night.

Additional tests provided included an elevated reticulocyte count of 200.4 x 109/L (7.45 %).

ADAMTS13 activity <5% and the ADAMTS13 inhibitor test reveals a result of >84.0U/ml (0-12), this is a positive result. Haptoglobins were low <0.24 with a reference range 0.45-2.05; The ADAMTS13 activity <10 % with an inhibitor most consistent with acquired TTP.
Plasma exchange (PEX) should be introduced as soon as possible. The surrounding clinical story including a relevant drug history will make it clear how the management plan might evolve.

ADDITIONAL COMMENT:
Thrombotic thrombocytopenic purpura (TTP) is a microangiopathic haemolytic anaemia classically characterized by the pentad of fever, haemolytic anaemia, thrombocytopenia, and renal and neurologic dysfunction. TTP results from either a congenital or acquired absence/decrease of the von Willebrand factor-cleaving protease ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13). Low levels of ADAMTS13 activity result in microthrombi formation, which leads to end-organ ischaemia and damage. The central nervous system and kidneys are the two most common organ systems affected by TTP.

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Acquired TTP is more common than the congenital type and is caused by autoantibodies targeting ADAMTS13. Antiplatelet drugs, immunosuppressive agents, HIV, oestrogen-containing contraceptives, and pregnancy are the most commonly listed triggers for ADAMTS13 autoantibody formation causing acquired TTP. The less common congenital form of TTP results from mutations to ADAMTS13. The deficiency of ADAMTS13 activity alone does not cause clinically apparent TTP. Individuals with hereditary ADAMTS13 deficiency remain asymptomatic until a triggering event such as an infection or pregnancy occurs. Risk factors for the development of inhibitory autoantibodies to ADAMTS13 are not clearly defined.

TTP is a rare disease; the exact prevalence is not clear. Studies cite incidences between 1 and 13 cases per million people depending on geographic location. TTP most often occurs after 40 years of age, but congenital forms can occur in children. TTP is more common in women with a 2:1 female to male predominance. The mortality in TTP without treatment is 90%, but this drops to a mortality of 10% to 15% with proper treatment. TTP is very rare in children. Other factors associated with a higher risk of TTP include female sex, African American descent, and pregnancy.

A good reference for appropriate testing and interpretation is 'The role of ADAMTS13 testing in the diagnosis and management of thrombotic microangiopathies and thrombosis' by Masias et al in Blood 2018 (https://doi.org/10.1182/blood-2018-02-791533).

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BCM 149:

DIAGNOSIS:

CLINICAL DETAILS:

NOTES: