ISSUE
36
MAY
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QSP Monthly

HORIBA Medical
NEWSLETTER OF THE QUALITY SLIDE PROGRAM

This issue

May Slides

Slide 1
See case study on right.

Slide 2
Emergency. Result of platelets flag "PLT aggregate" on the analyser. **Note:** Large platelet clusters at the edges of slide visible (on the wall of red blood cells). **Expert's comments:** Platelet count represents a minimum-suggest citrated platelet count to confirm.

Slide 3
Emergency. **Note:** Smear a little scratched.

Slide 4
Clinical Haematology unit. Thrombocytosis. Erythrocytosis. Marked platelet anisocytosis associated with thrombocytosis. Set evocative of a myeloproliferative syndrome. JAK2 gene mutation (V617F)? **Expert's comment**: Platelet anisocytosis, polycythemia. In favour of a Vaquez-type MPS (Primary Polycythemia). Low MCV and low MCHC => Bleeding Hypersegmented PNNs => Under hydroxyurea?

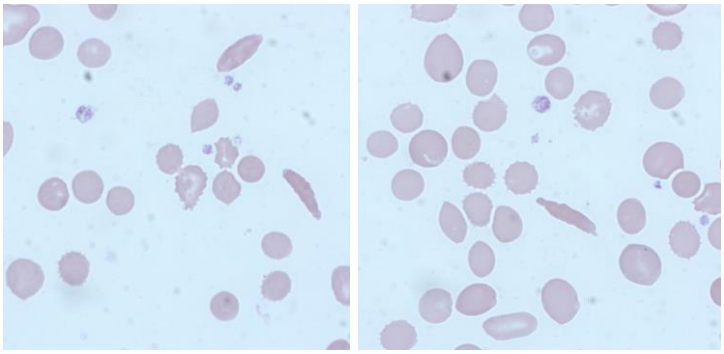
Slide 5
Clinical Haematology unit. Hyperleukocytosis. Neutropenia (hyposegmented and hypogranulated neutrophils). Blastosis (+++). Aniso-poikilocytosis(++).

Slide 6
Microcytic anisocytosis. Hypochromia. Microcytes(++). Anisocytosis(++). Poikilocytosis(++). Elliptocytes/ovalocytes(++). Hypochromia(++). **Expert's comments**: Iron deficiency.

Monthly Digital Case Study Presentation
May 2023, Slide 1

FBC Results

WBC **7.2** (10^3/mm3)
RBC **2.64** (10^6/mm3)
HGB **8.2** (g/dL)
HCT **25.4** (%)
MCV **96** (fL)
MCH **31.1** (pg)
MCHC **32.3** (g/dL)
PLT **354** (10^3/mm3)
Neutrophils **24.6**%
Lymphocytes **55.2**%
Monocytes **10.1**%
Eosinophils **8.7**%
Basophils **1.4**%



Anaemia and sickle-shaped red blood cells are consistent with the clinical and biological context.

Clinical Details

01 May 2023, Male (age not given).

Slide Information

Clinical context: "Result of the study of haemoglobin by HPLC/Electrophoresis: Presence of S haemoglobin (31.2%) in favour of homozygous sickle cell disease, known transfused or SBeta/Thal-Beta composite haemoglobinopathy.

Expert Comment

Aniso-poikilocytosis(++). Sickle cell(++). Echinocytes(++). Hypochromic (+) RBCs. Reticulocytosis (9%). Erythroblastosis (23%). The white blood cells seem +/- altered, - Apoptosis is clearly visible, especially on certain images of Polynuclear Neutrophils, - And the presence of Echinocytes(++). The whole is very evocative of an "old" sample. Anaemia and sickle-shaped red blood cells are consistent with the clinical and biological context. Suggest family investigation and/or a molecular biology study is recommended."

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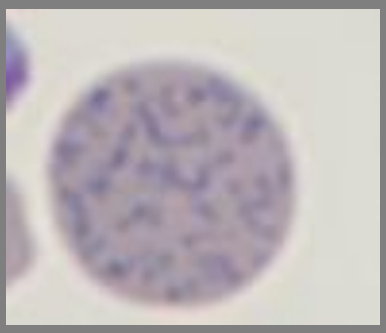
Cell Quiz

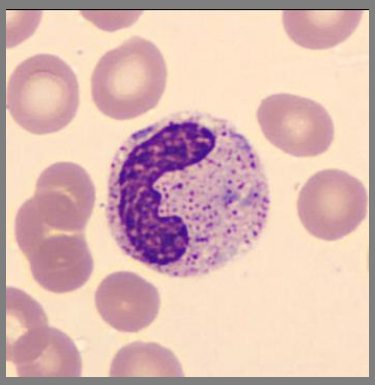
What feature can be seen in the red cell?



A) Basophilic Stippling
B) Bitten Out cell
C) Cabot's ring

Last Month's Quiz

Name this cell and what can be seen inside it?



Right answer:
The neutrophil cell above contains Döhle bodies. These are small, round or oval, pale blue–grey structures usually found at the periphery of the neutrophil. They consist of ribosomes and endoplasmic reticulum.

Haemoglobinopathies - An introduction

Haemoglobinopathy is a clinical term that describes a **group of blood** disorders that affect red blood cells. Haemoglobinopathies are inherited disorders of globin, the protein component of haemoglobin (Hb). Mutations in genes coding for the globin proteins which alter protein output produce the thalassaemia syndromes. Mutations in the globin genes that lead to abnormal proteins are called variant Hbs. Haemoglobinopathies are the commonest genetic defect worldwide with an estimated 269 million carriers. Certain populations are particularly at risk of having a haemoglobinopathy, for example, in south-east Asia, there are 90 million carriers, about 85 million in sub-Saharan Africa, and 48 million in the West Pacific.

Common types are:

- Sickle cell disease
- Thalassaemia
- Haemoglobin C disease
- Haemoglobin E disease

Symptoms

The **insufficient production of haemoglobin** or the abnormal structure results in poor functioning of red blood cells. Consequently, **anaemia** can occur. In very serious cases of a haemoglobinopathy, patients may experience the following symptoms:

- Weakness and fatigue due to anaemia
- Shortness of breath
- In children, there can be problems relating to growth

Diagnostic work up

The detection and characterisation of a haemoglobinopathy involves 3 stages. Starting with full blood count and film, special haematological tests (HPLC) and possible DNA testing.

Initial detection of Haemoglobinopathies, especially thalassaemia begins with the FBC. The first indication for a thalassaemia would be a low MCV/MCH. As iron deficiency would give the similar MCV/MCH results, ensure iron deficiency is excluded/treated. Subsequent FBC results giving the same low MCV/MCH would be suggestive of a thalassaemia. Note that MCV can also be raised in some other conditions, such as B12 and folate deficiency. HIV patients using nucleoside analogues can also develop a low MCV. A HbS carrier could be missed if only an MCV/MCH is used at initial screening.

It is common practice to make a blood film on samples with a reduced MCV/MCH. A blood film would give a clue as to the presence of sickle disease (HbS) or an unstable Hb. Observations may be stippling and target cells. Although these features are not exclusively associated with haemoglobinopathies, they are helpful to build the patient picture.

Tips

- The RDW (red cell distribution width) measures the coefficient of variation for the MCV. It tends to be higher in iron deficiency but not in thalassaemia's and so can give an indication to which is more likely. However, particularly during pregnancy, it is not unusual to find both could be present.
- Some haemoglobinopathies, particularly HbS, will have a normal MCV and normal MCH and would be missed if the full blood count is used as a screening test.

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- Since the foetal to adult β globin switch is not usually complete until about 6 months of life, it is difficult to detect β thalassaemia in the neonate based on the full blood count. However, a paediatric haematologist experienced in looking at blood films and dealing with thalassaemia might make a reasonable guess, based on the haematologic parameters and red blood cell changes in the blood film whether these changes are suggestive of thalassaemia. The presence or absence of Beta Thalassaemia in the parents of the child is very important In determining the probability of thalassaemia.
- At risk pregnancies which have not been monitored by prenatal diagnosis (a late diagnosis or the couple is not interested in prenatal testing), it is important to keep some of the cord blood for DNA testing to be undertaken.

The haemoglobinopathy screen using HPLC is used in conjunction with clinical details and results of the FBC to identify Haemoglobin variants and thalassaemia. All Hb variants detected are confirmed by a secondary method. This screen is commonly used in antenatal screening, pre-operative screening and to provide HbS% for transfused sickle cell patients.

HPLC can be used to detect levels of the normal constituents of Hb, HbA, HbA2 and HbF as an increase in HbA2 is indicative of Beta Thalassaemia or the presence of HbH (tetramers of beta globulin). HbH can be seen in the cytoplasm of red cells after staining with Brilliant cresyl Blue with the affected red cells displaying a characteristic Golf Ball appearance. The Prescence of abnormal Hb variants are detected by HPLC due to their abnormal transition time.

Special haematology tests are requested once a haemoglobinopathy is suspected based on family history and/or full blood count. Often these tests are ordered by requesting a "thalassaemia or haemoglobinopathy screen".

Test	What it detects	What it means
HbEPG	Electrophoresis of globin proteins. Different techniques possible from gel or membrane- based kits to HPLC. Abnormal bands apart from the usual HbA, HbF and HbA2 peaks can be detected.	Gives indication of the HbA2 level but more importantly identifies if there are any variant Hbs – particularly Hbs such as HbE and HbS.
HbA_2	Globin electrophoresis and quantitation of the HbA2 peak. Different techniques from membrane or column-based kits but more universally suited HPLC are used.	A raised HbA2 is the key parameter indicating the presence of β thalassaemia. Hbs can raise the HbA2 but this must be a rare event. A borderline normal/raised HbA2 could indicate silent β thalassaemia. A low HbA2 is also important to note as this might indicate δ thalassaemia.
HbF	Globin electrophoresis and quantitation with different methods available.	A slightly raised HbF to 2–3% (normal is <1% in an adult) might indicate heterocellular HPFH or a subtle pointer to an underlying silent β thalassaemia. HbF levels 5% and above are more likely to be due to δβ thalassaemia or. In the case of δβ thalassaemia you would expect the HbA2 level to be low.

Other special haematological tests are available, particularly when investigating the more uncommon variant Hbs. These include tests for oxygen affinity, haemoglobin stability and detection of methaemoglobin. Mass spectrometry has been used to characterise various variant Hbs.

The third stage of testing would include DNA testing. Methods include mutation analysis (usually PCR based), DNA scanning and DNA sequencing. See next issue for more information on Haemoglobinopathies.

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