ISSUE
15
July
2021

HORIBA Medical

QSP Monthly

NEWSLETTER OF THE QUALITY SLIDE PROGRAM

This issue

Last Month's Slides

Side 1
Presence of blasts/granular blasts.
Probable AML 1

Slide 2
Oncology Patient.
Monomorphic hyperlymphocytosis.
Presence of atypical lymphoblasts with irregular nuclei.

Slide 3
See case study on right

Slide 4
Nothing abnormal noted

Slide 5
Nothing abnormal noted

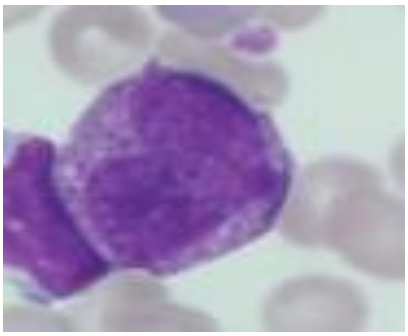
Slide 6
Nothing abnormal noted

Monthly Digital Case study
June 2021

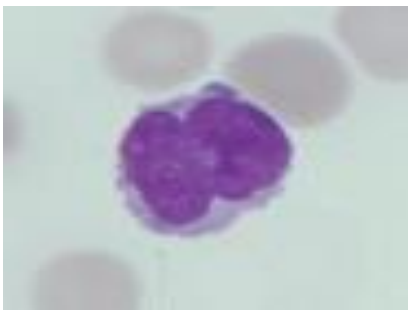
Presentation
Male 77 years old.

FBC Results

WBC	46.9 (10^3/mm3)
RBC	3.13 (10^6/mm3)
HGB	9.9 (g/dL)
HCT	29.4 (%)
MCV	94 (fL)
MCH	31.6 (pg)
MCMH	31.6 (g/dL)
PLT	36 (10^3/mm3)
Neutrophils	22.9 %
Lymphocytes	72.0 %
Monocytes	1.0 %
Eosinophils	0.0 %
Basophils	0.0 %



Myelocyte



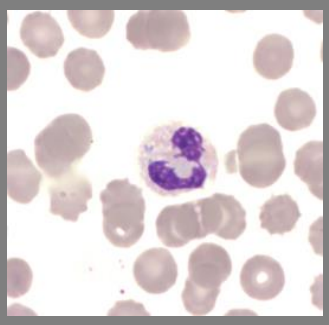
Sezary Cell

Slide review
Probable lymphoproliferative disorder
Immunophenotyping of circulating Lymphocytes.
Sezary cell?
T-cell lymphoproliferative syndrome, presence of granular lymphocytes. Cancerous T-cells. Called Sezary cells, characterised by an abnormally shaped nucleus, known as Cerebriform (looks like a brain).

QSP Quality Slide Program 2.0

Cell Quiz

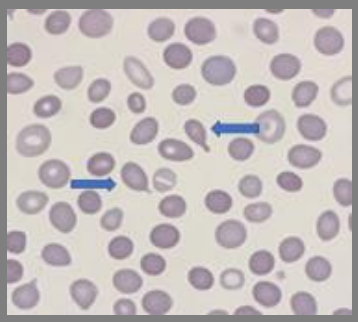
From the following choose the description to best describe the prominent feature in the displayed Neutrophil



A) Left Shift
B) Neutro-Toxic Granulation
C) Hypo Granular
D) Activated

Last Month's Cell Quiz

Look at the slide & choose the correct red cell from the multiple choice below:



Answer:

C) Tear drop cell

Teardrop cells, or dacrocytes, are red blood cells with one round end and one pointed end. Teardrop cell formation may be multifactoral but appears to involve the distortion of the red cells as they pass through the marrow or spleen. They are a type of poikilocyte which can be seen in the morphology of patients with:

- Beta thalassaemia,
- Myelofibrosis,
- Leukaemia,
- Megaloblastic anaemia,
- Haemolytic anaemia

Paroxysmal Nocturnal Haemoglobinuria (PNH)

PNH is a rare haematopoietic stem cell disorder (estimated to affect 1-10 cases per million) first recognised as a distinct clinical entity in the 1800's. PNH is clinically heterogenous with some patients having a disease which presents with overwhelming intravascular haemolysis whilst in other bone marrow failure is the predominant feature. PNH can occur at any age of life and is a life long condition. Approximately half of patients with PNH will die as direct consequence of their disease, many others are transfusion dependent for decades.

In 1882, Paul Strübing recognised PNH as being distinct from Paroxysmal Cold Haemoglobinuria and March Haemoglobinuria after observing the episodic occurrence of haemoglobinuria in a 29 year old cartwright. He recognised that the red cells had been haemolysed in the blood vessels rather than the kidney i.e. few or no intact red cells in the urine. He discovered that haemoglobinuria was only present during the night (apart from upon wakening), therefore the term PNH was derived. He hypothesized that symptoms of PNH were a consequence of the abnormal sensitivity of PNH red cells to acidosis resulting for the accumulation of CO2 during sleep. This hypothesis was used to develop the first diagnostic test for PNH – the Ham's test (acidified serum lysis test) - developed by Thomas Ham in the 1930's and to the pivotal role complement plays in the lysis if red cells in PNH.

In the 1930 – 1960's it was discovered that PNH red cells were particularly sensitive to complement mediated haemolysis which focussed research onto the exact reasons for this. It was also recognised that PNH red cells from the same person have different susceptibilities to complement mediated haemolysis with one cohort being many times more sensitive than the other. Investigation of the PNH red cell structure identified that there was a deficiency of multiple cell surface proteins and these were identified as being GP1 anchored proteins. In 1993 the exact genetic mutation was identified by Dr Kinoshita et al as being a mutation of the PIGA gene. The two most important proteins in terms of inducing complement-mediated intravascular haemolysis are CD55 (decay accelerating factor [DAF]) and CD59 (membrane inhibitor of reactive lysis – [MIRL]). There is no evidence that GPI anchored proteins other than CD55 and CD59 contribute to the clinical pathophysiology of PNH, therefore can be used as diagnostic markers.

Although PNH is a clonal disease, it is not a malignant disease in that the clone does not expand exponentially and does not invade sites outside of the bone marrow. Some patients have only a very small clone of PNH affected cells which can only be detected by high sensitivity flow cytometry whilst other patients have large clones which all haematopoiesis is derived from the mutant stem cell. PIGA is located on the X chromosome, therefore only one mutational event is necessary to produce PNH cells, as males only have 1 X chromosome and females only 1 of the 2 X chromosomes is active in somatic tissues. Aplastic Anaemia is associated with PNH and it is possible that PNH is as a consequence of Aplastic Anaemia. Patients with PNH suffer from a degree of bone marrow failure which exacerbates anaemia if there is ongoing intravascular haemolysis.

Paroxysmal Nocturnal Haemoglobinuria

As free haemoglobin is released into the serum, it irreversibly binds to Nitric Oxide which decreases its concentration. Nitric Oxide is a key molecule in homeostasis and the lower levels led to smooth muscle dysfunction, platelet activation and other consequences. This results in severe disabling abdominal pain, dysphagia, profound lethargy and in men erectile dysfunction.

Approximately 50% of all PNH patients have thrombotic events the most common of which are venous thrombosis of the Liver (Budd-Chiari syndrome), abdomen (portal, mesenteric, spleen) and the brain. The risk of developing thrombosis is correlated to the proportion of PNH clones and the degree of intravascular haemolysis.

Diagnosis of PNH

Basic Tests:
FBC, Reticulocytes
Urine Haemoglobinuria
Urine Haemosiderin
Advanced Test
Fluorescent Aerolysin (FLAER) flow cytometry assay
Flow Cytometry for CD55 and CD59

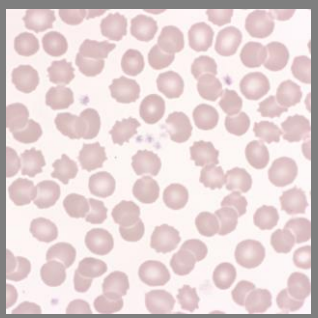
Treatment:
Treatment depends upon the severity of the disease with the most severe benefitting from Eculizumab which blocks the activation of complement, thereby protecting the PNH cells from haemolysis.

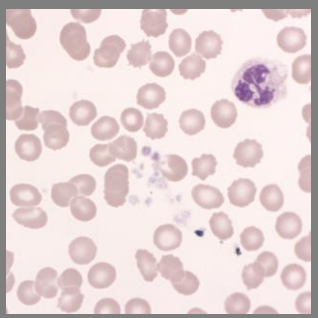
Blood transfusion is given to maintain red cell levels in the less severe forms of PNH along with Folic acid, Iron and Iron chelating treatments.

The only cure for PNH is a Allogenic Bone Marrow Transplant.

MORPHOLOGY TIP

EDTA tubes are routinely used in laboratories to prevent clotting and sample storage. Prolonged storage of samples in EDTA can, however, cause morphological changes to the blood sample, which are visible on a blood film. Erythrocytes are particularly effected. The timing between sample collection and analysis is therefore crucial. EDTA effects over 24 hours can include crenation, spiculation and the formation of echinocytes or Burr cells. Artefact can also occur, making a clear examination of the blood film difficult.
Identifying storage related changes is therefore important, so that artefactual changes are not misinterpreted as true pathological findings.
If in doubt, ALWAYS check the date and time taken on a sample suspected of showing EDTA changes.





Red cells showing EDTA deterioration

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