Morphology: An Overview.

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Learning Objectives

01

Morphology Basics

Why we make blood films, how we review them and what they can tell us.

02

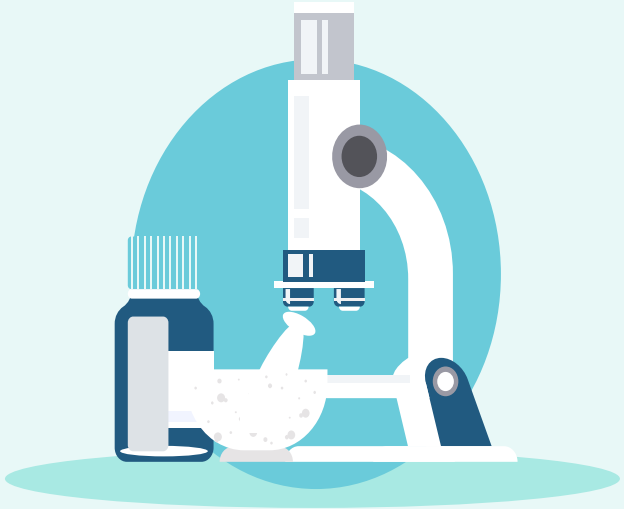
Key cell Features

An overview of features you'd expect to see on a blood film and their value.

03

Cases

Four interesting cases to provide context.



Morphology Basics.

What is a Blood Film?

Smear of Blood

Peripheral blood is spread on a glass side to produce a thin layer of cells.

Cells examined under a microscope

Light microscopy used to look at cell structures.

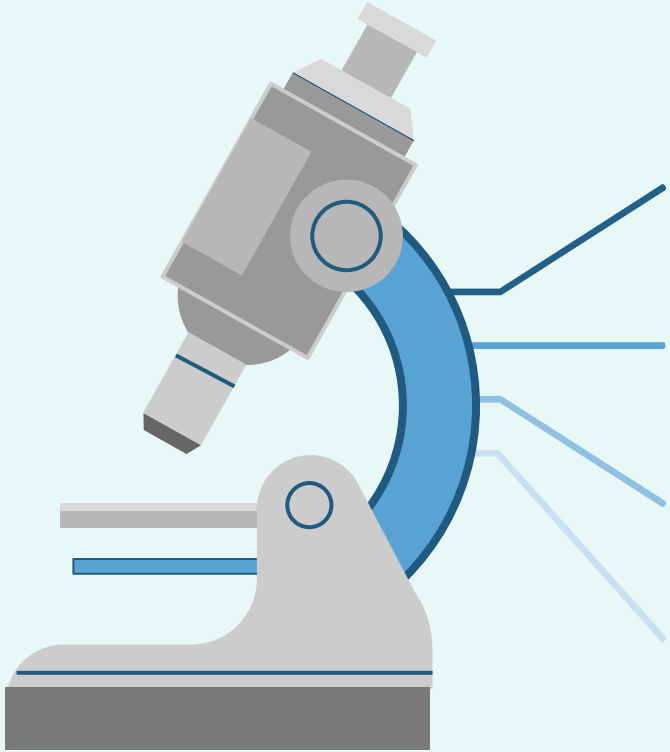
Manual or Automated

Blood film examination can be fully automated, or done entierly by hand!

Skill is Essential

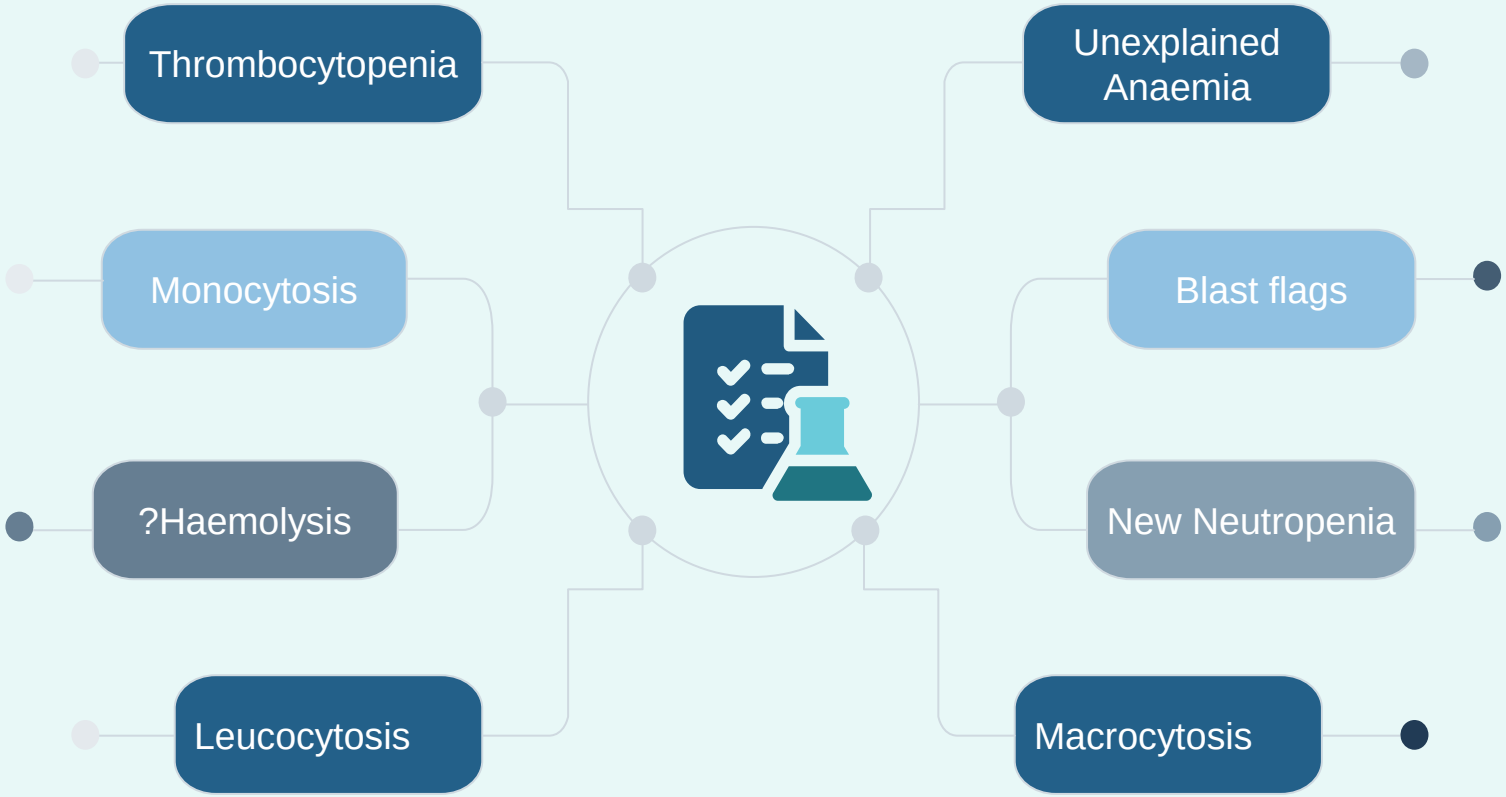
The value of a blood film is dependant on the skill of the examiner.

Why are Blood Films Important?



1. **Qualitative Data**
   Analyser can't tell us about shape changes etc.
2. **Confirms the Analyser Findings**
   Neutropenia, platelet counts etc.
3. **Contradicts the Analyser!**
   Blasts and monocytes, target cells and lymphs
4. **Diagnostic Features**
   Characterstic patterns which are diangostic.

Why do we Request Blood Films?



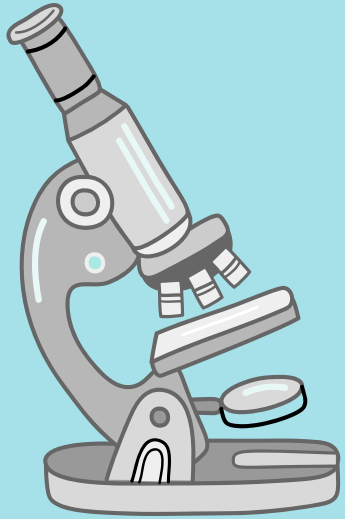
Stains and Microscopes

Giemsa Stain

Contains Azure A (thiazde). This is a metachromatic dye.

May-Grunwald

Contains contains eosin (acidophillic) and methylene blue (basophillic) dyes.



Magnification

Blood films are examined at x10 and x60 magnificantion.

Oil Emmersion

Oil has a higher refractive index than air, so more light is captured by the lens

Key Cell Features

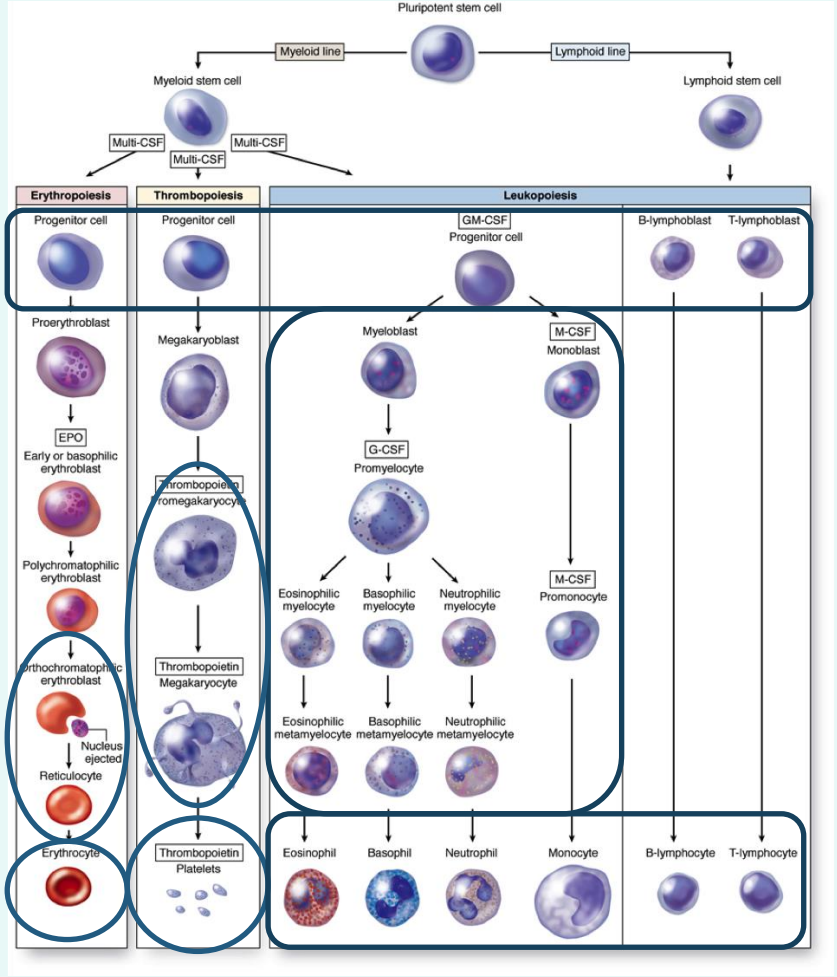
Blood Cells.

All blood cells are made in the bone marrow, but only a few enter into peripheral blood (PB).

Megakaryocytes are extremely uncommon in PB.

Red cell precursors are common in anaemia

These cells are normal in peripheral blood.



Key Cell Features

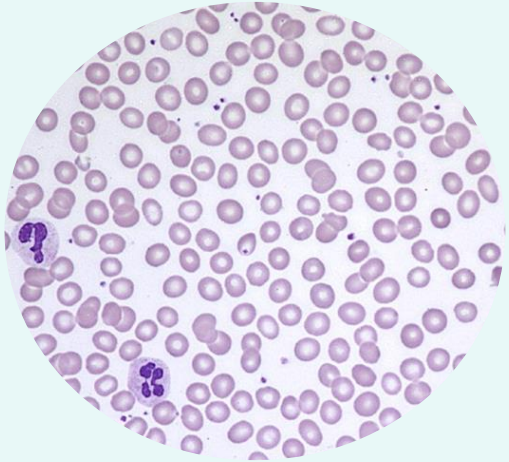
Blasts are always concerning in peripheral blood!

Myeloid precursors can be present in PB for many reasons- not all maligant!

Key Cell Features

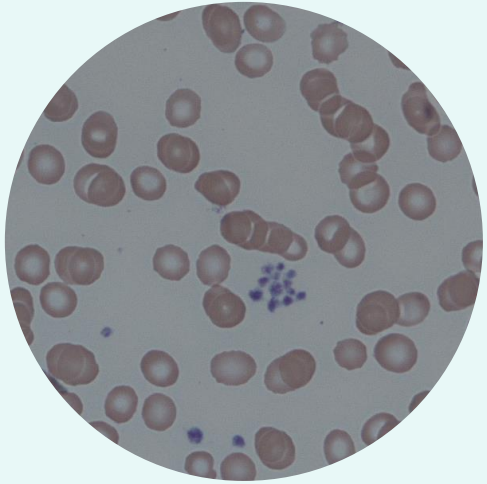
Platelets: Key Changes.

Normal



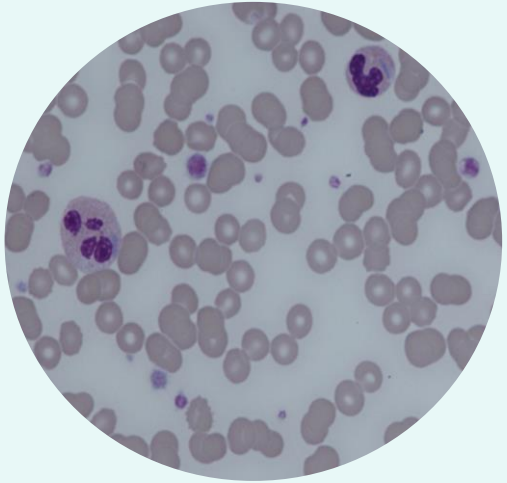
Platelets should be small and evenly distributed

Platelet Clumping



In vitro anomaly only

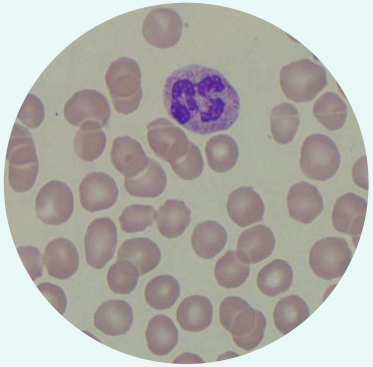
Giant Platelets



May Hegglin Anomaly
Glanzmann's thrombocythaemia
Immune thrombocytopenia

Red Cell Anaemia

Key Cell Features

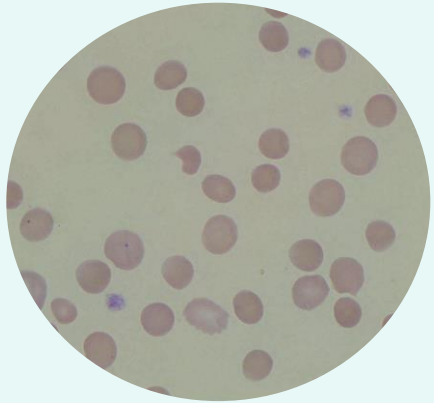


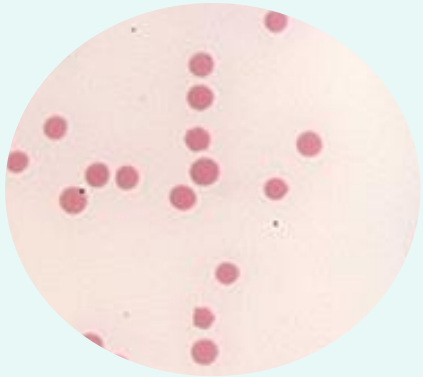
Macrocytic

MCV >105fl
B12/Folate Deficiency
Myelodysplasia
Alcoholism

Normocytic: Haemolytic

Premature erythrocyte destruction
Auto/Allo immune
Microangiopathy



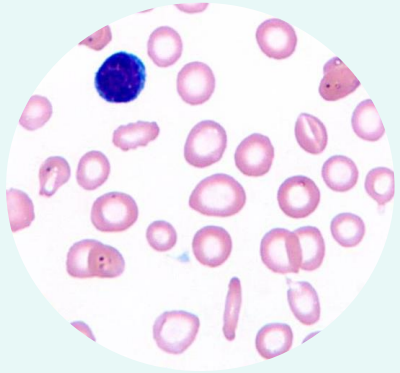


Normocytic: Cytopenic

Reduced production or loss
Aplastic anaemia
Bleeding

Microcytic

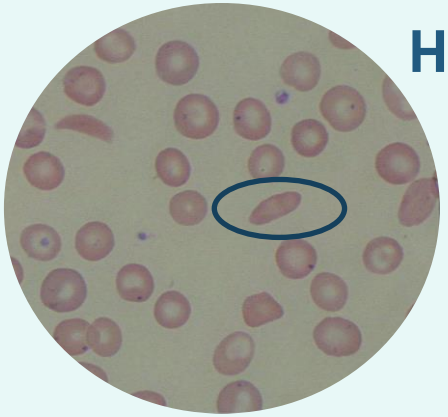
MCV <80fl
Iron deficiency
Haemoglobinopathies



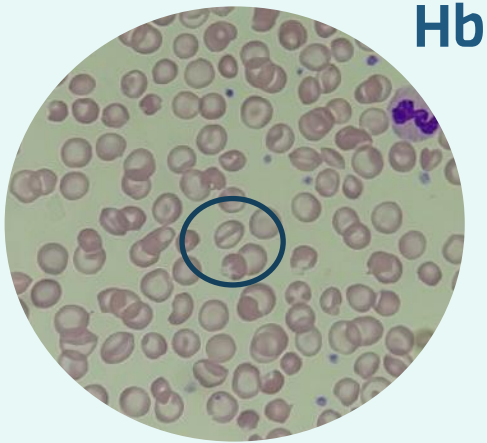
Key Cell Features

Red Cells: Haemoglobinopathies

HbS/S



HbC/C

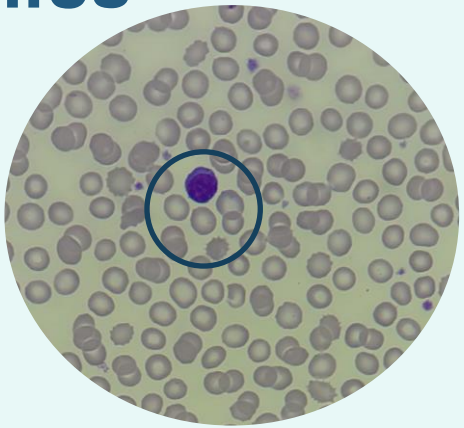


HBOPs

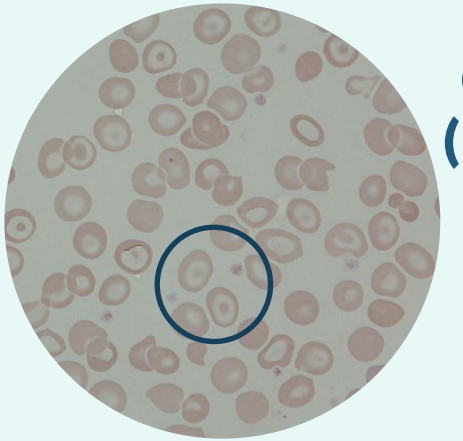
Key features:

- Abnormal shapes
- Target cells
- Microcytosis with normal Hb (trait)

β-Thal (Trait)

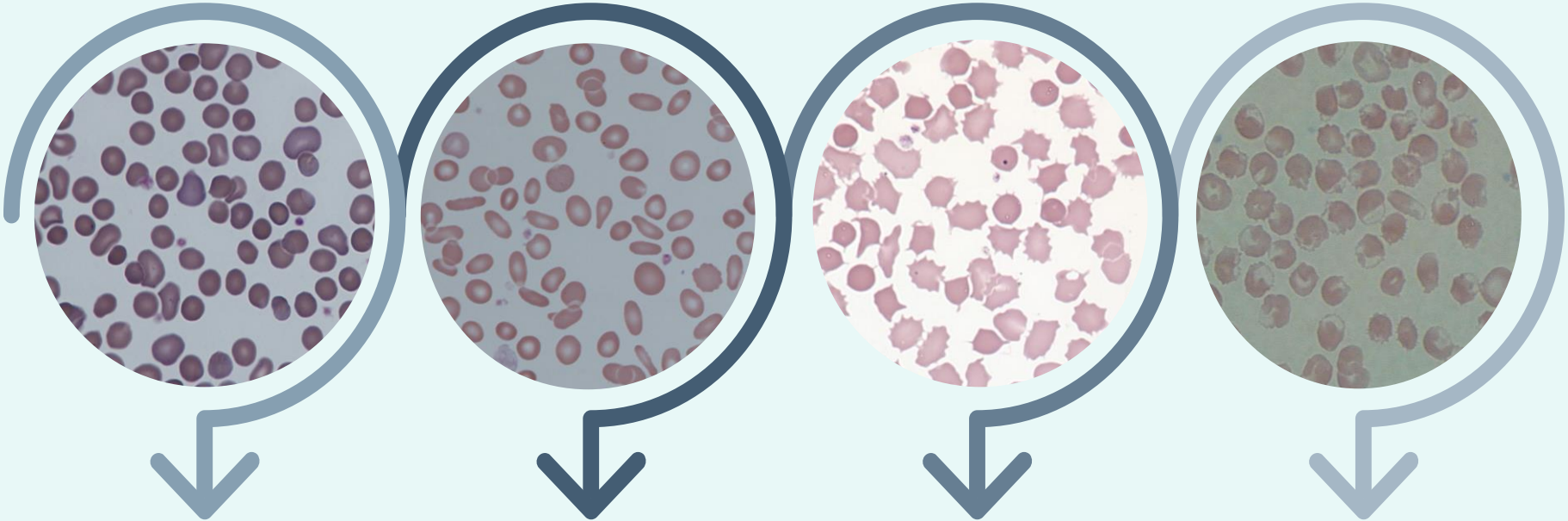


α-Thal (Major)



Key Cell Features

Red Cells: Inherited Abnormalities



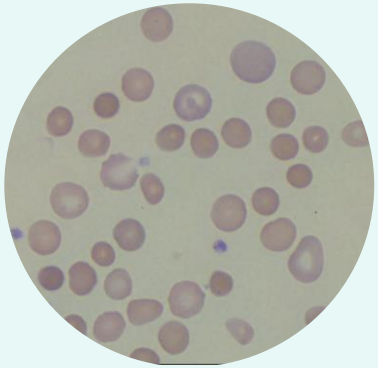
Hereditary Spherocytosis

Hereditary Elliptocytosis

Pyruvate Kinase Deficiency

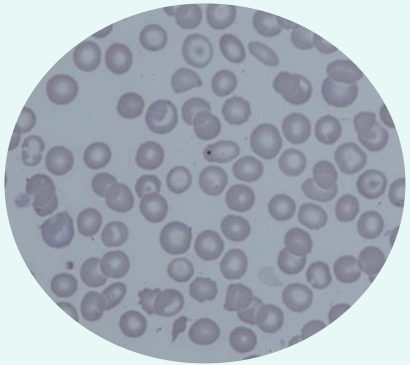
G6PD Deficiency

Red Cells: Other Aquired Abnormalities



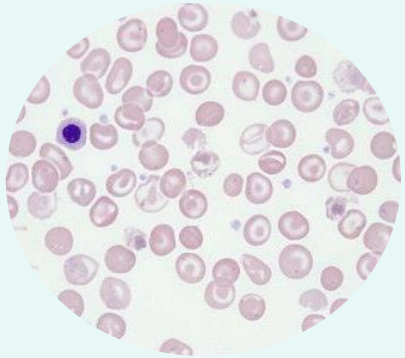
Autoimmune Haemolysis

Spherocytes
Reticulocytes
Nucleated red cells



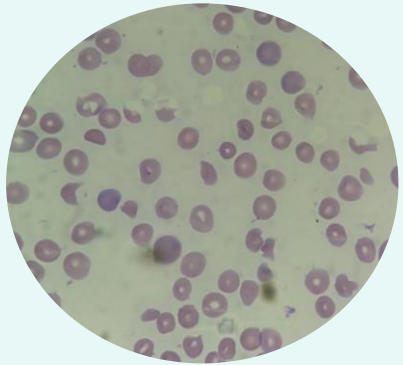
Splenectomy

Howell Jolly Bodies
Bite cells
Target cells



Liver Failure

Target cells.
Stomatocytes
Increased precursors



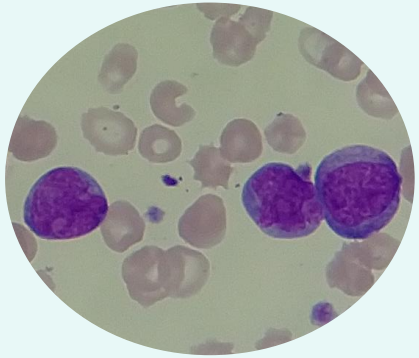
Microangiopathic Haemolysis

Fragments
Thrombocytopenia (often)
Polychromasia

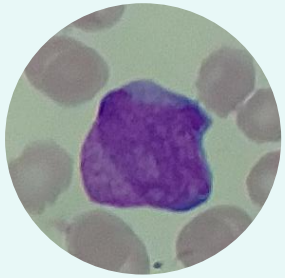
Key Cell Features

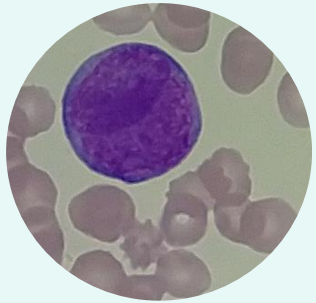
The wonderful world of Blasts!

"Classical" Blasts



Acute Promyelocytic Leukaemia



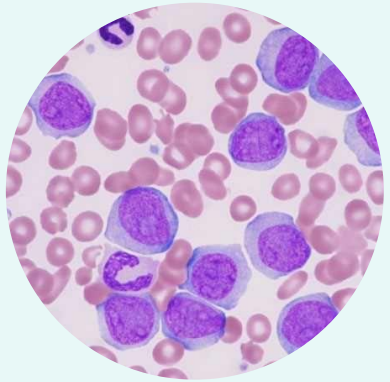


Blasts actually very variable!

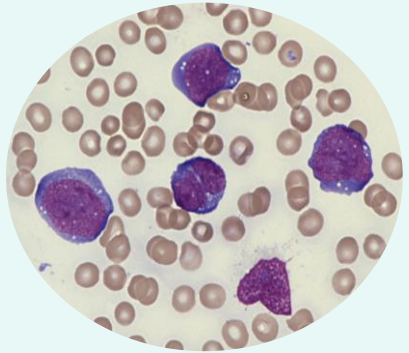
Key features to look for:

- Open chromatin
- Large cells
- Dark cytoplasm

Monoblasts

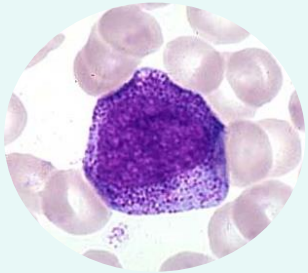


Lymphoblasts



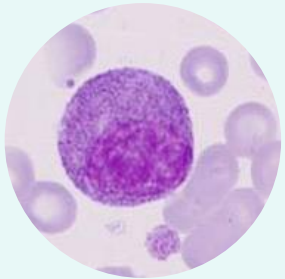
Key Cell Features

Granulocytes: Development



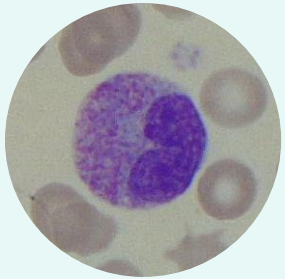
Promyelocyte

Large nucleaus with nucleoi and primary granules.



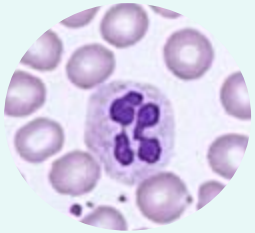
Myelocyte

Large nucleus with condensed chromatin and secondary granules



Metamyleocyte

Developed nuclear lobation, defined secondary granules

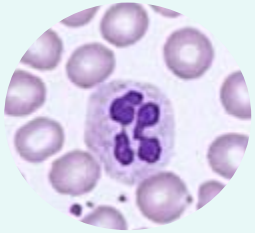


Neutrophil

Complete lobe maturation with no primary granules

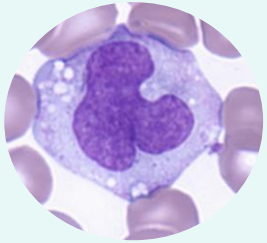
Normal Granulocytes

Key Cell Features



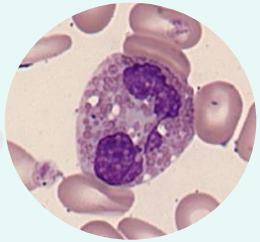
Neutrophil

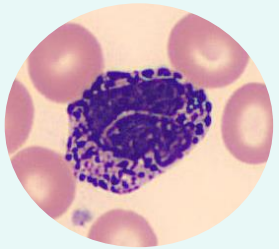
Monocytes



Eosinophil

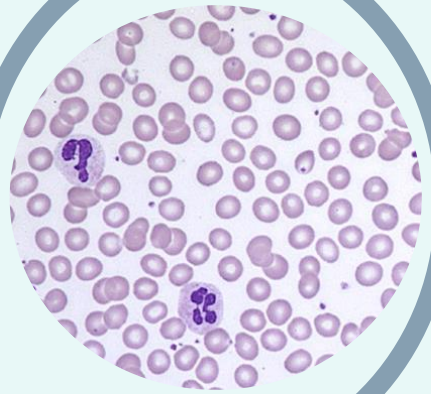
Basophil



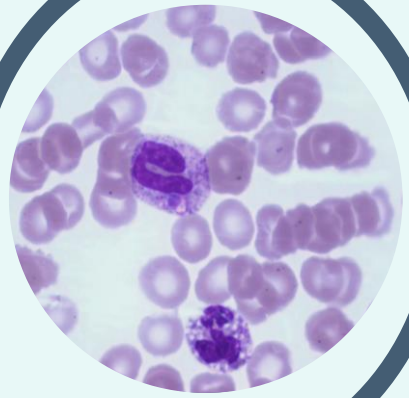


Granulocytes: Non-malignant

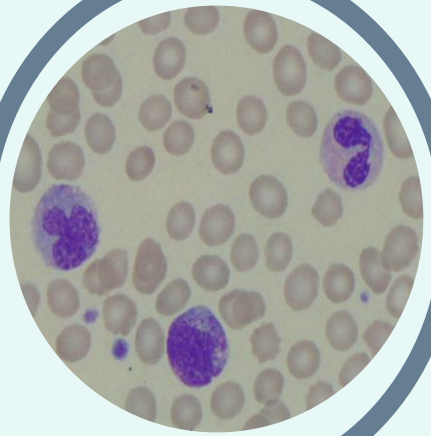
Key Cell Features



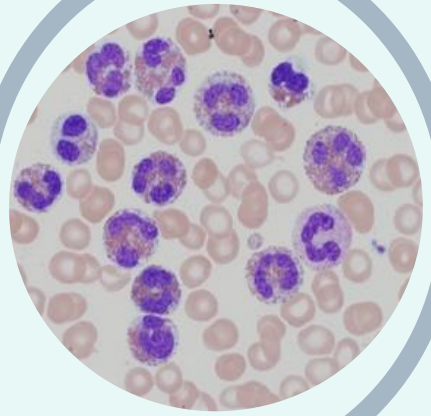
Normal



Bacterial Sepsis



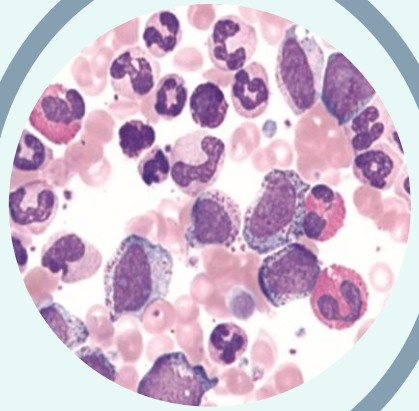
GCSF



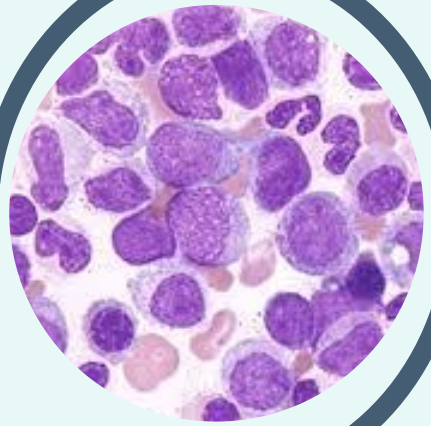
Parasitic Infection

Granulocytes: Malignant

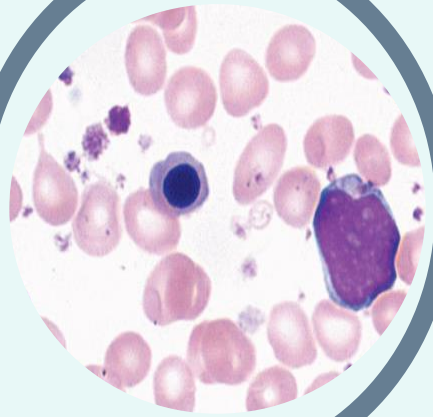
Key Cell Features



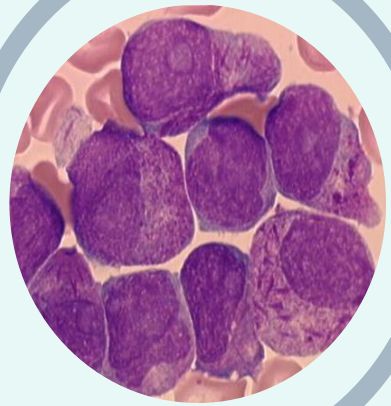
Chronic Myeloid Leukaemia



Chronic Myelomonocytic Leukaemia



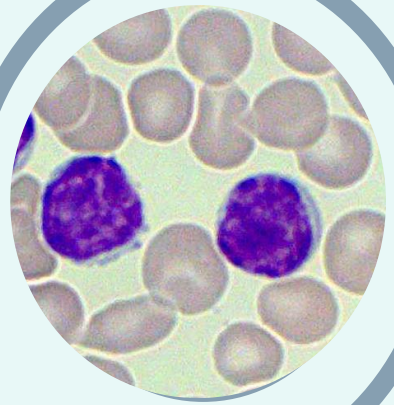
Myelofibrosis



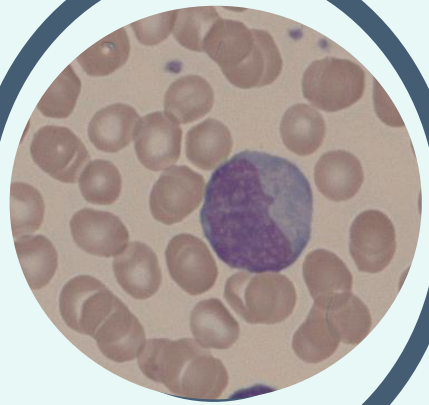
AML with Auer Rods

Lymphocytes: Non-malignant

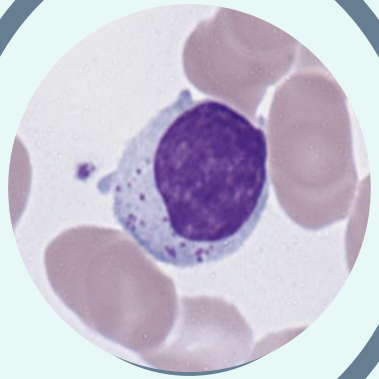
Key Cell Features



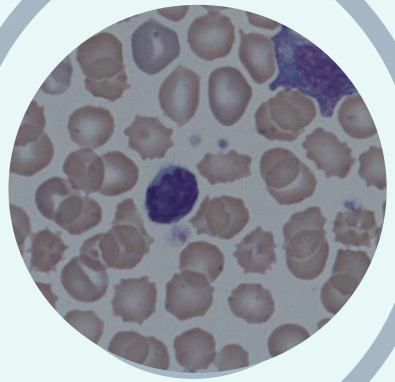
Normal



Infectious Mononucleosis



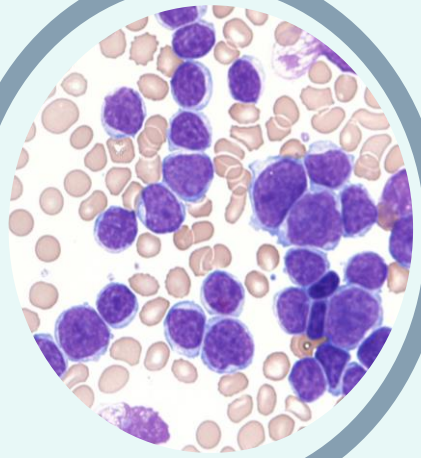
Large granular lymphocytes



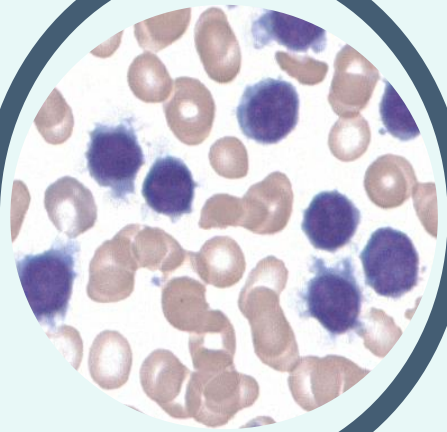
Neonatal Lymphocyte

Lymphocytes: Malignant

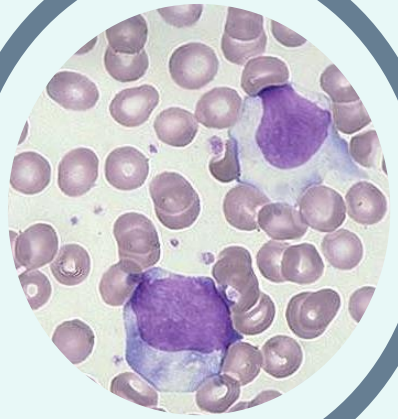
Key Cell Features



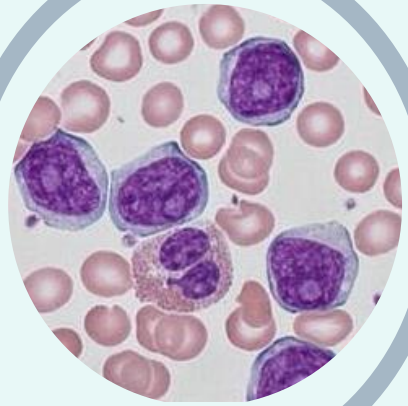
Chronic Lymphocytic Leukarmia



Hairy Cell Leukaemia



Lymphoma



Prolymphocytic Leukaemia

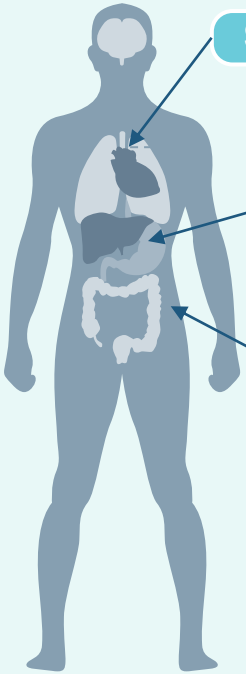
Cases

Case 1

Case 1: Presentation

73 year old Male

- Past medical history: **Chronic Lymphocytic Leukaemia (CLL).**
- Presented to their clinic appointment with deterioration.
- The patient was admitted with transformation to high grade lymphoma.
- He developed tumour lysis syndrome and was given Rasburicase.



Shortness of Breath

Loss of Appetite

New lymphadenopathy

Suddenly, the patients haemoglobin dropped and he began experiencing severe symptoms of anaemia.

Case 1: FBC Results

What's abnormal about this FBC?

22/02/2022 17:45 Blood
Request Reason : unwell.

HB	48	g/L	(130 to 170)	Auth
WBC	18.2	10*9/L	(4.0 to 11.0)	Auth
PLT	236	10*9/L	(150 to 410)	Auth
RBC	1.40	10*12/L	(4.50 to 5.50)	Auth
HCT	0.126	L/L	(0.400 to 0.500)	Auth
MCV	90.3	fL	(83 to 101)	Auth
MCH	34.3	pg	(27.0 to 32.0)	Auth
MCHC	380	g/L	(315 to 345)	Auth
RDW	15.1		(11.6 to 14.0)	Auth
MPV	7.2	fL	(7.5 to 11.2)	Auth
Neutrophils	17.1	10*9/L	(2.0 to 7.0)	Auth
Lymphocytes	1.0	10*9/L	(1.0 to 3.0)	Auth
Monocytes	0.1	10*9/L	(0.2 to 1.0)	Auth
Eosinophils	0.0	10*9/L	(0.00 to 0.5)	Auth
BAS	0.0	10*9/L	(0.0 to 0.1)	Auth
Neutrophil-Lymphocyte Ratio	17.1	Ratio		Auth
Automated Nucleated Red Count	^0.5	10*9/L	(0.0 to 0.1)	Auth
uncorrected WBC	^18.2	10*9/L		Auth
Retics(Absolute count)	91.10	10*9/L	(50 to 100)	Auth
Immature Retic Fraction	0.74	ratio	(0.16 to 0.36)	Auth
Reticulocyte %	^6.5	%		Auth

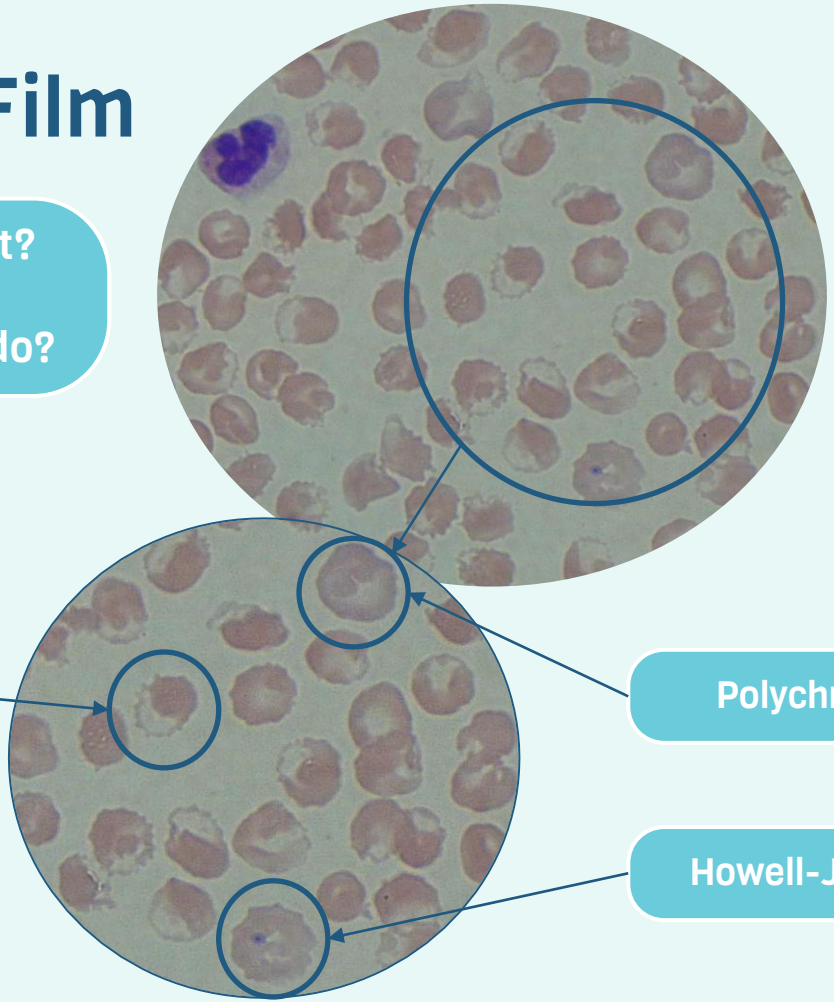
Would you make a blood film on this sample?

Case 1

Case 1: Blood Film

What do these features suggest?

What further testing should we do?



Blister/Hemi Ghost Cell

Polychromasia

Howell-Jolly Body

Case 1

Case 1: Further Investigation

Biochemistry

Tot. Bilirubin	78	umol/L	(	0	to	21	)	Auth
Total Protein	50	g/L	(	60	to	80	)	Auth
Albumin	27	g/L	(	35	to	50	)	Auth
Alk.Phos.	95	U/L	(	30	to	130	)	Auth
Urate	30	umol/L	(	200	to	430	)	Auth
LDH	850	U/L	(	135	to	225	)	Auth

What's wrong with this patient?

Enzymopathy Investigation

G6PD Assay (Pointe Scientific) (Acc No: 6PS22B0439137)		Req:20/02/2022 11:37:00		
G6PD (assay)	0.5	↓	(6.6 - 13.8)	[IU/g Hb]

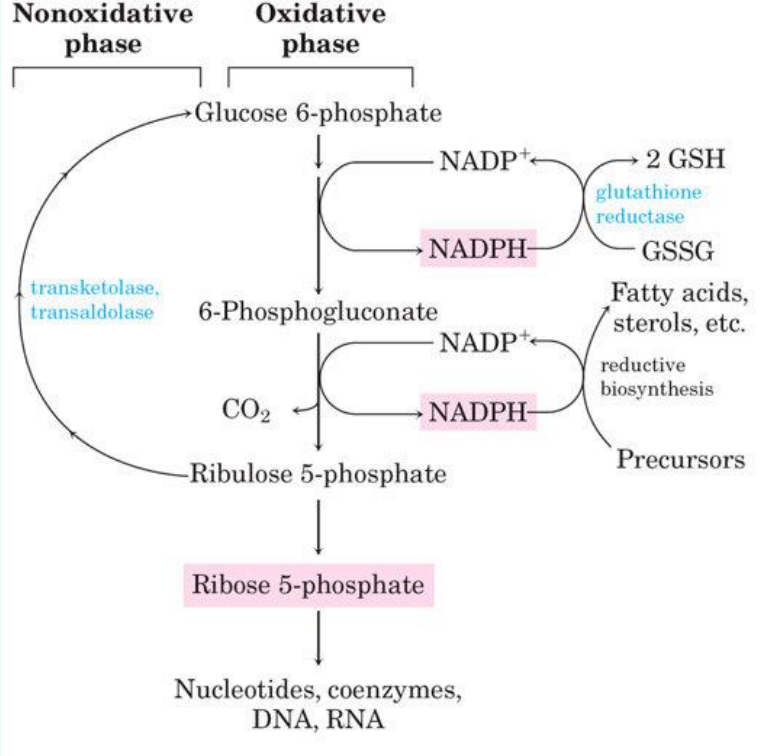
Case 1

Case 1: Diagnosis and Conclusions

- Rapid, acute haemolysis in the presence of oxidative chemicals.
  - Such as **Rasburicase.**
  - **Also Fava beans!**
- X-Linked Recessive Disorder.
  - Can affect XX individuals
- Most common in African, Middle Eastern and South Asian people.
  - But anyone can be affected!

This patient's results would need to be actioned urgently by a clinician!

This patient has G6PD Deficiency

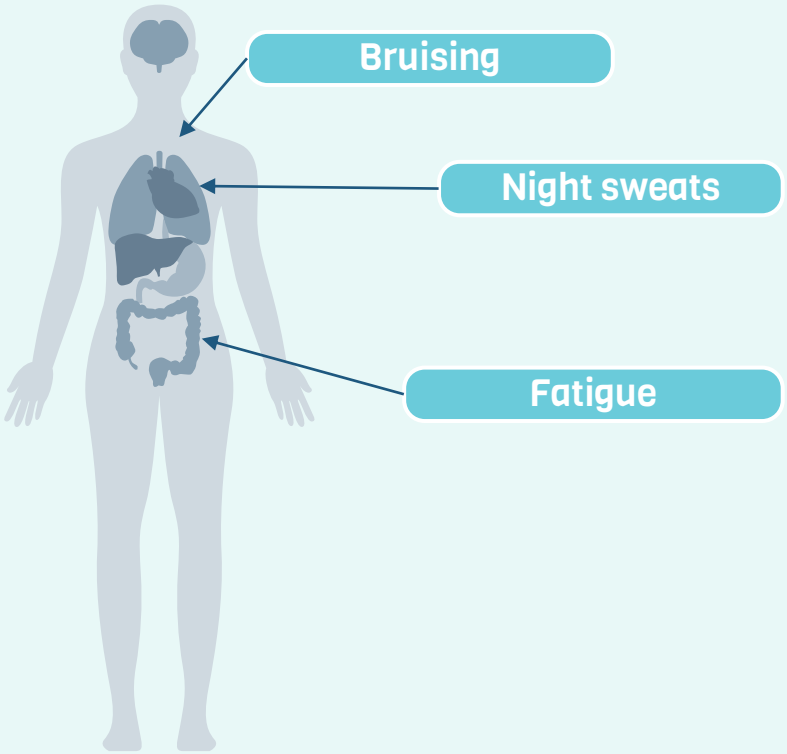


Case 2

Case 2: Presentation

59 year old Female

- **Past medical history**: None.
- She has 2 children and runs an online vintage clothing shop.
- She is extremely fatigued with unusual bruising and poor wound healing.
- The GP requests a generic panel of testing including an FBC.



Case 2

Case 2: FBC Results

What's abnormal about this FBC?

bruising ++. NONE \NONE \ Date received: 28/01/2021 R
Specimen No : HQ836651R Haematology <PgUp/PgDn> for more

28/01/2021 08:45 Blood
Request Reason : bruising ++. NONE

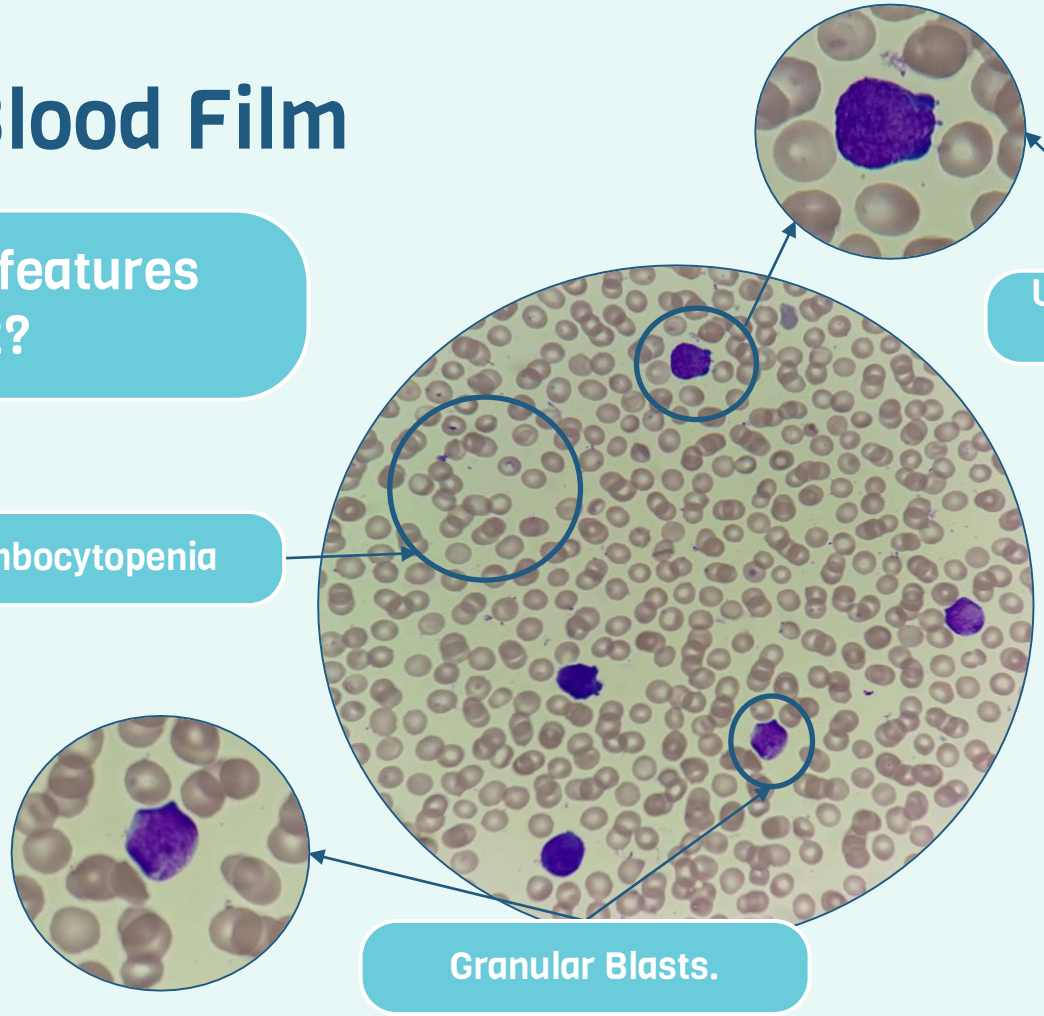
HB	115	g/L	(120 to 150)	Auth
WBC	7.4	10*9/L	(4.0 to 11.0)	Auth
PLT	4	10*9/L	(150 to 410)	Auth
RBC	3.67	10*12/L	(3.80 to 4.80)	Auth
HCT	0.333	L/L	(0.360 to 0.460)	Auth
MCV	90.6	fL	(83 to 101)	Auth
MCH	31.3	pg	(27.0 to 32.0)	Auth
MCHC	346	g/L	(315 to 345)	Auth
RDW	15.3		(11.6 to 14.0)	Auth
MPV	10.0	fL	(7.5 to 11.2)	Auth
Neutrophils	^0.6	10*9/L	(2.0 to 7.0)	Auth
Neutrophils.....	0.5	10*9/L	(2.0 to 7.0)	Auth
Lymphocytes	^3.0	10*9/L	(1.0 to 3.0)	Auth
Lymphocytes.....	2.8	10*9/L	(1.0 to 3.0)	Auth
Monocytes	^3.8	10*9/L	(0.2 to 1.0)	Auth
Monocytes.....	0.2	10*9/L	(0.2 to 1.0)	Auth
Eosinophils	^0.0	10*9/L	(0.00 to 0.5)	Auth
Eosinophils.....	0.0	10*9/L	(0.00 to 0.5)	Auth
BAS	^0.0	10*9/L	(0.0 to 0.1)	Auth
Basophils.....	0.1	10*9/L	(0.0 to 0.1)	Auth

Would you make a blood film on this sample?

Case 2: Blood Film

Case 2

What do these features suggest?



Undifferentiated Blasts.

Thrombocytopenia

Granular Blasts.

Case 2

Case 2: Prelimnary Diagnosis

- **This patient likely has AML.**
- Granular blasts makes Myeloid likely.
- Leukaemia causes ↓ in other cells.
  - The bone marrow is making cancer cells.
  - This contributes to symptoms.

This AML in particular needs to actioned urgently- why?

Case 2

Case 2: Further Investigation

What kind of AML does this patient have?

WRGL Sample No:		Specimen Type:	**Blood**
Date Received:	**29/01/2021**	Date Reported:	**01/02/2021**

Referral Reason: AML, few bi-lobed with some granules, exclude APL. For urgent PML-RARA FISH, G-banded analysis and AML molecular panel.

FISH: **nuc ish(PML,RARA)x3(PML con RARAx2)[70/100]**

Interphase FISH analysis was performed on a direct culture of this blood sample using the Cytocell *PML/RARA* dual fusion probe combination, which detects the classic 15;17 translocation seen in APL. The presence of a *PML-RARA* [t(15;17)] rearrangement, with a standard dual fusion signal pattern, was detected in 70 of the 100 cells examined (this result was reported on the phone to Dr Belsham on 29/01/21).

The *PML-RARA* [t(15;17)] rearrangement defines a category of AML in the 2017 revision of the WHO classification of haematopoietic neoplasms and, according the 2017 ELN recommendations for the diagnosis and management of AML (Döhner *et al.*, Blood 2017; 129(4):424-447) and the MRC AML trial protocol (Grimwade *et al.*, Blood 2010; 116:354-365; data from patients aged 16-59 years), is associated with a good prognosis.

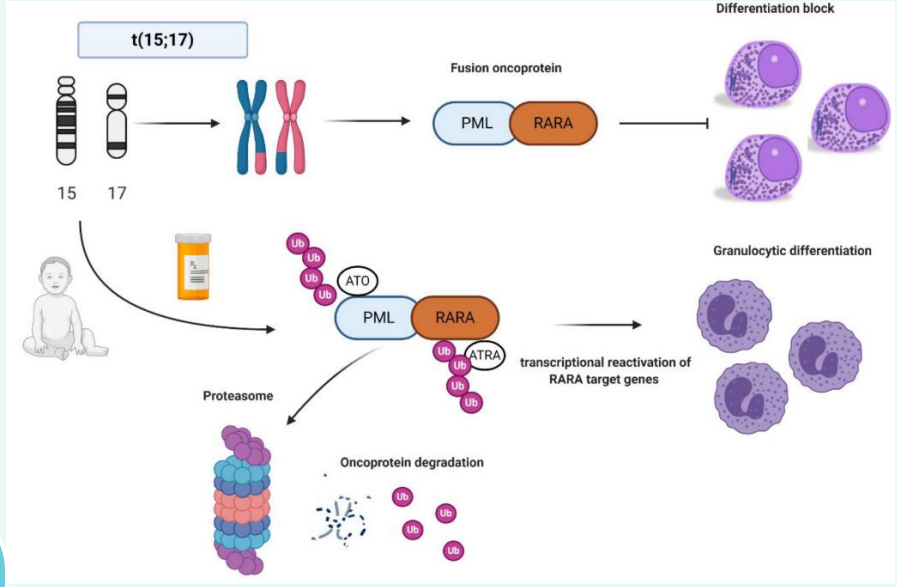
G-banded analysis from cultures of this blood sample will also be performed, as well as RT-PCR to determine the exact breakpoints of the 15;17 rearrangement; results from these tests will be reported in due course. The AML molecular panel and the myeloid NGS panel will be undertaken on the separate bone marrow sample received 29/01/21 (our ref. W2101371).

Case 2

Case 2: Diagnosis and Conclusions

- APML is a type of AML.
- APML is especially dangerous.
  - Patients can develop **Disseminated Intravascular Coagulation.**
- APML can be treated with Retinoic Acid (ATRA) and Arsenic.
  - APML has a 90% remission rate with treatment.

This patient has Acute Promyelocytic Leukaemia

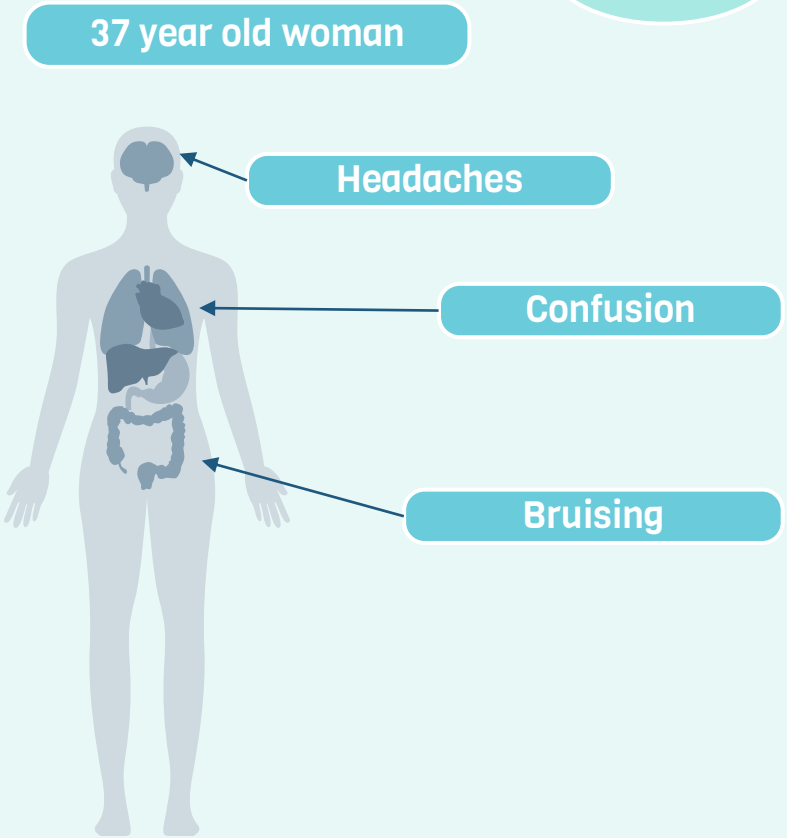


ATRA is nontoxic, so it's better to start and be wrong!

Case 3

Case 3: Presentation

- Patient presents to Accident and Emergency.
- **Past Medical History:** None.
- Patients symptoms are non-specific.
- The following is requested.
  - Full blood count
  - U&Es, Liver Function, Bone Profile.



Case 3

Case 3: FBC Results

11/11/2021 01:27 Blood
Request Reason : unwell.

HB	57	g/L	(120 to 150)	Auth
WBC	16.0	10*9/L	(4.0 to 11.0)	Auth
PLT	12	10*9/L	(150 to 410)	Auth
RBC	1.72	10*12/L	(3.80 to 4.80)	Auth
HCT	0.164	L/L	(0.360 to 0.460)	Auth
MCV	95.6	fL	(83 to 101)	Auth
MCH	33.2	pg	(27.0 to 32.0)	Auth
MCHC	347	g/L	(315 to 345)	Auth
RDW	17.7		(11.6 to 14.0)	Auth
MPV	11.0	fL	(7.5 to 11.2)	Auth
Neutrophils	10.7	10*9/L	(2.0 to 7.0)	Auth
Lymphocytes	4.0	10*9/L	(1.0 to 3.0)	Auth
Monocytes	1.2	10*9/L	(0.2 to 1.0)	Auth
Eosinophils	0.1	10*9/L	(0.00 to 0.5)	Auth
BAS	0.1	10*9/L	(0.0 to 0.1)	Auth
Neutrophil-Lymphocyte Ratio	2.7	Ratio		Auth
Monocyte Distribution Width	^21.7		(2.0 to 19.9)	Auth
Automated Nucleated Red Count	^1.2	10*9/L	(0.0 to 0.1)	Auth
uncorrected WBC	^16.0	10*9/L		Auth
Retics(Absolute count)	309.77	10*9/L	(50 to 100)	Auth
Immature Retic Fraction	0.74	ratio	(0.10 to 0.36)	Auth
Reticulocyte %	^18.0	%		Auth

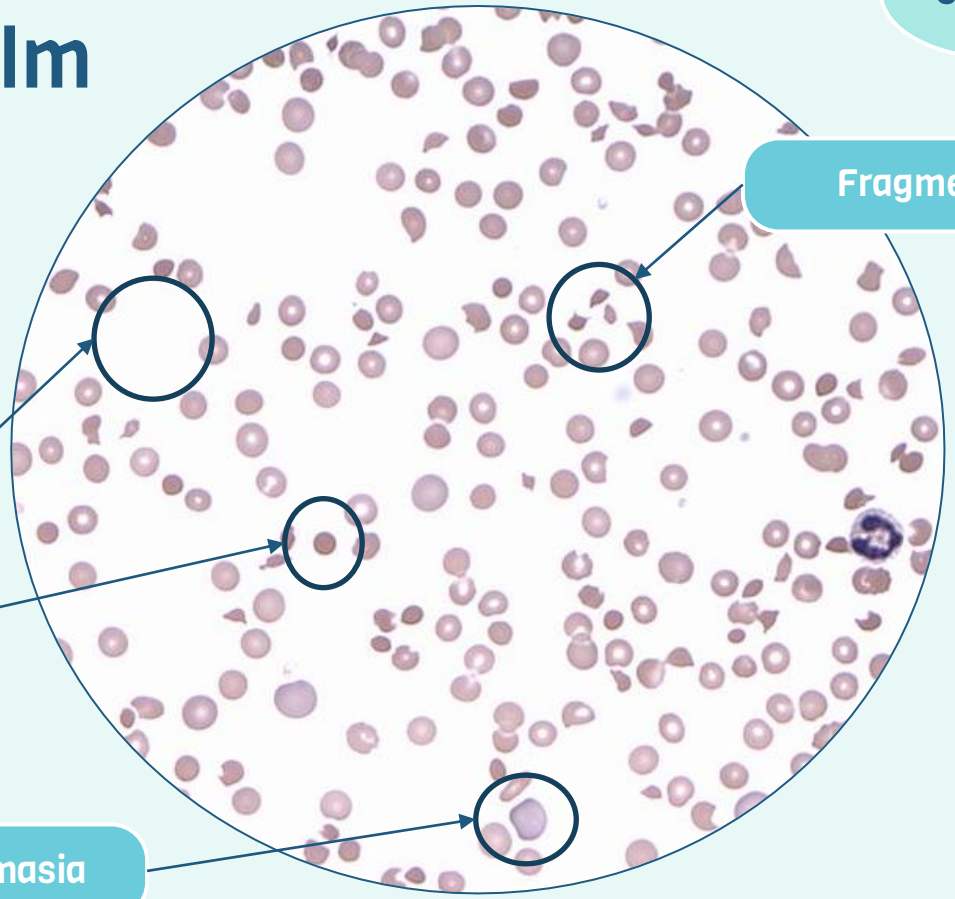
What's abnormal about this FBC?

Would you make a blood film on this sample?

Case 3

Case 2: Blood Film

What do these features suggest?



Fragments

Thrombocytopenia

Spherocytes

Polychromasia

Case 3

Case 3: Prelimnary Diagnosis

- **This patient has a haemolytic anaemia.**

- Haemolysis is characterised by:
  - Normocytic, normochromic Red Cells.
  - **Red Cell Damage**: Fragments, spherocytes
  - Increased red cell precursors.
- Haemolysis can be:
  - **Intravascular:** Contents released into plasma.
  - **Extravascular:** Shortened lifespan.

How should we action these results?

What other results should we check?

Case 3

Case 3: Further Investigations

Tot. Bilirubin	37	umol/L	(3 to 20)	Auth
Total Protein	71	g/L	(61 to 79)	Auth
Albumin	39	g/L	(35 to 50)	Auth
Alk.Phos.	85	U/L	(30 to 130)	Auth
C-Reactive Protein	78	mg/L	(0 to 7)	Auth
Alanine Transaminase	37	U/L	(7 to 35)	Auth

High sensitivity troponin I	232.3	ng/L	(0 to 17.4)	Auth

Comments :
A positive hs Troponin result in a patient with
a coagulopathy may be unreliable. Please interpret
this result in conjunction with clinical findings.
See hyperlink from this test in ICE for new pathway.
Result phoned to Matthew Jenkins @0401hrs 11.11.21 pc

LDH	4332	U/L	(225 to 425)	Auth

Corona Virus PCR	NOT detected
COVID19 Request Received	COVID-19 Request Received
Request Received in Lab	Request Received in Lab
CDR report	Auth
^SARS-CoV-2 Negative Not Detected	

Test	Patient Result
Haptoglobin	↓↓
DAT	Negative

What's the likely diagnosis?

INR.....	1.0		(0.8 to 1.2)	Auth
APTR.....	0.7		(0.8 to 1.2)	Auth

Comments :
? activated sample - please repeat.

Fibrinogen.....	3.8	g/L	(1.5 to 3.5)	Auth
Plasma D-Dimer.....	2.35	ugFEU/ml	(0 to 0.5)	Auth

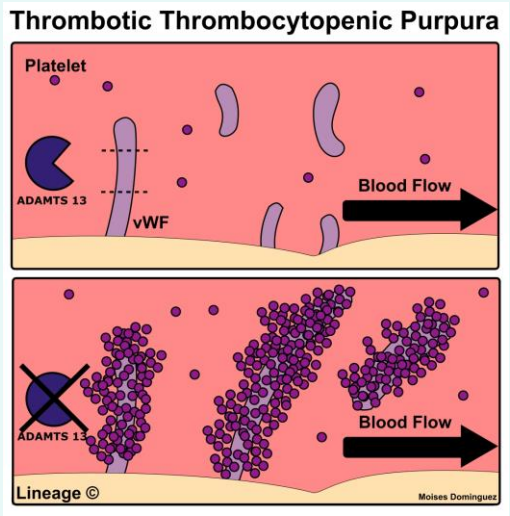
Case 3: Diagnosis and Conclusions

Case 3

- Confirmed by ADAMTS13 testing.
- ADAMTS13 cleaves vWF.
  - Ultra large vWF.
  - Highly procoagulant environment.
- Treatment is **Total Plasma Exchange**.
- TTP is rare, but the typical demographic is young women.

This patient has Thrombotic Thrombocytopenia Purpura (TTP)

11/11/2021 22:45 Blood				
Request Received in Lab	SRLH Request	Auth Received in Lab		
ADAMTS13 activity (Fret)*	7.4	Auth	IU/dL	(60 to 146)
anti ADAMTS13 assay*	42.0	Auth	%	(Up to 6.1)



Treatment should be started BEFORE TTP is confirmed.

Without treatment, TTP has a >90% mortality rate!

Case 4

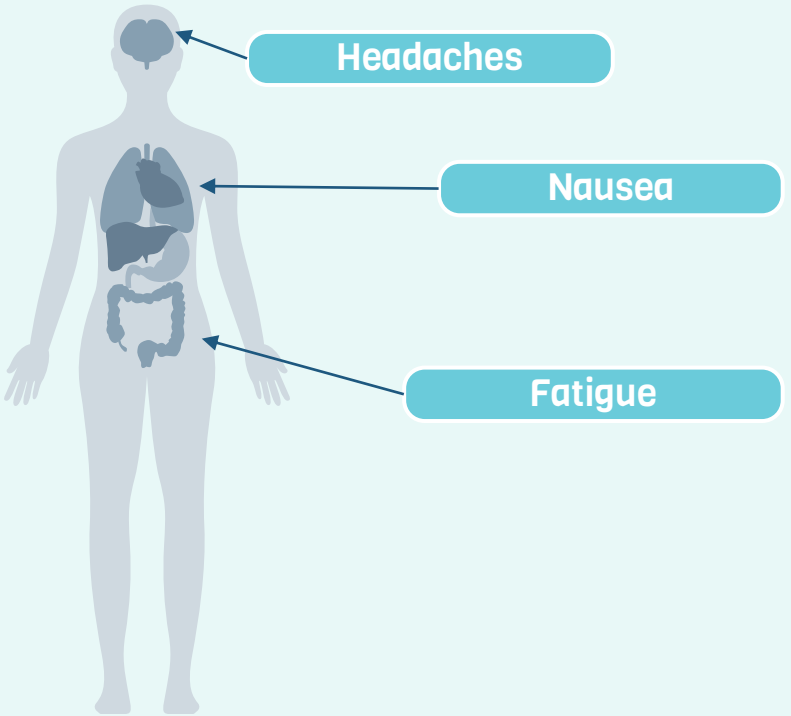
Case 4: Presentation

25 year old woman

- Patient presents to her GP.
- **Past medical history:** Thalassemia trait and previous (negative) investigations for coeliac.

- Current Investigations:
  - **COVID-19 PCR:** Negative .
  - **Home Pregnancy Test:** Negative.
  - **Travel History:** None.

- Currently self isolating as works on a children's ward.
- GP asks for a FBC and general Biochemistry tests.



Case 4: FBC Results

What's abnormal about this FBC?

HB	142	g/L	(130 to 170)	Auth
WBC	13.9	10*9/L	(4.0 to 11.0)	Auth
PLT	84	10*9/L	(150 to 410)	Auth
RBC	4.52	10*12/L	(4.50 to 5.50)	Auth
HCT	0.416	L/L	(0.400 to 0.500)	Auth
MCV	92.1	fL	(83 to 101)	Auth
MCH	31.4	pg	(27.0 to 32.0)	Auth
MCHC	341	g/L	(315 to 345)	Auth
RDW	12.7		(11.6 to 14.0)	Auth
MPV	10.9	fL	(7.5 to 11.2)	Auth
Neutrophils	11.8	10*9/L	(2.0 to 7.0)	Auth
Lymphocytes	0.9	10*9/L	(1.0 to 3.0)	Auth
Monocytes	1.2	10*9/L	(0.2 to 1.0)	Auth
Eosinophils	0.0	10*9/L	(0.00 to 0.5)	Auth
BAS	0.1	10*9/L	(0.0 to 0.1)	Auth

Would you make a blood film on this sample?

Case 4

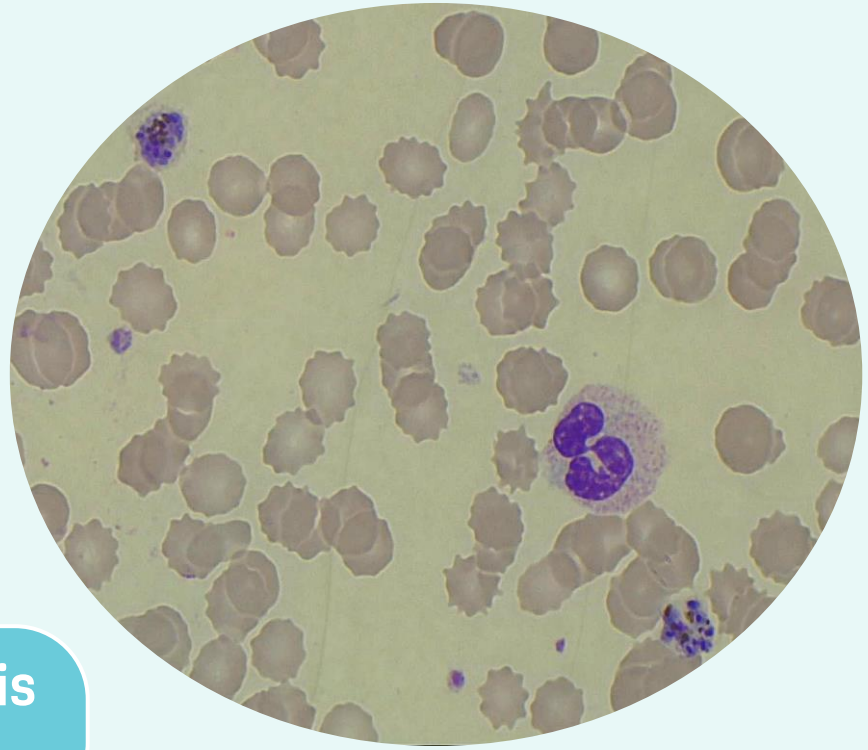
Case 4: Blood Film Report.

- A newly qualified Band 5 BMS reviewed the film and gives the following report.
- The film was placed on the Clinical Authorising Queue because of the thrombocytopenia.

```
FILM
LTG Comments :
  some stomatocytes,
  platelets on film agree with auto count,
  visual differential agrees with automated,
```

Case 4: Worsening Clinical Status

- In the meantime the patient has been admitted to ACEM with worsening symptoms.
- The lab is asked to re-review the film for a haemolysis and fragmentation.
- The film is reviewed by a senior BMS.



What's wrong with this patient?

Case 3: Diagnosis and Conclusions

Case 4

- The patient travelled 5 months ago to Nairobi to visit family.
- The patient **did not** have symptoms on her return.
- The patient was treated as an inpatient and made a full recovery.

This patient has plasmodium Ovale.

p.Ovale **and** ***p.Vivax*** **can reside asymptomatically in the liver for up to 40 months.**

Known as Hypnozoites.

But how was it missed?

Case 4: How was it missed?

Clinical Suspcion

Malaria was not suspected, so extented testing wasn't requested.

Low Parasitemia

The patient's parasitemia was <1%. The BMS may not have reviewed enough fields.

Inexpereince

BMS may have mistaken parasites for giant platelets. Standard staining does not highlight parasite pigments well.

It's easier to miss than you'd think!

Conclusions

1

Complex

There are many things to consider when reviewing a blood film!

2

Diagnostic

Information in blood films can be diagnostic

3

Context

Consider patient information as, age, gender etc. can aid diagnosis

4

Expereince

Expereince is essential, especially in subtle conditions

Thanks!

Do you have any questions?

Jennifer.mills@porthosp.nhs.uk
023 92 28 5774