Can We Still Learn From the Blood Film?

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What Might a Blood Film Help Us Do?

Blood film morphology may

• Help you detect a pre-analytical or analytical error in the output of an automated instrument
• Confirm or explain an instrument flag
• Tell you something that no automated instrument can yet tell you

Pre-analytical Errors Identifiable by Blood film Morphology

• Platelet count is too low
• Platelet count is too high
• White cell count is too low
• White cell count is too high
• Hb and/or red cell indices are factitious

Platelet Count is Too Low

• Badly taken or poorly mixed specimen – partly clotted

Bain Interactive Haematology
Imagetable Blackwell Science
Platelet Count is Too High

A Pre-analytical Error in an Antenatal Patient

- A routine antenatal blood count sent to the laboratory by a midwife doing a domiciliary visit gave a highly abnormal blood count
  - WBC 11.96 x 10^9/l
  - Hb 10.7 g/dl
  - MCV 82.6 fl
  - RDW 20.6
  - Platelet count 1539 x 10^9/l
A Pre-analytical Error in an Antenatal Patient

- What is the most likely diagnosis?

- The ‘diagnosis’ was artefactual laboratory results
  - These resulted from blood being left on the dashboard of a hot car
  - The blood film simulates hereditary pyropoikilocytosis or burns
White Cell Count is too Low

- Very old specimen

White Cell Count is too High

- Contamination, e.g. with subcutaneous fat

Analytical Errors Identified by Blood Film Morphology

A blood count was done on a patient being followed up for neutrophilia.
A blood count was done on a patient being followed up for neutrophilia.

The laboratory said the neutrophil count was $0.8 \times 10^9/\text{l}$.

The clinician requested a bone marrow.

The laboratory said the neutrophil count was $0.8 \times 10^9/\text{l}$.

The bone marrow was done.

Better to think first.

It was absolutely normal.

The bone marrow was done.
White Cell Count is Too High

- Non-lysed red cells

Analytical Errors in Platelet Counts

“Thrombocytopenia”

Body of film

“Thrombocytopenia”

Tail of film
A 43-year-old man with no history of bleeding or bruising
Routine blood count performed before angiography
Automated platelet count on an impedance-based instrument: 108 x 10^9/l
Blood count otherwise normal
A blood film was examined

Repeat blood sample
Film prepared with no exposure to anticoagulant — no phagocytosis
After brief exposure to EDTA occasional neutrophils showed satellitism and phagocytosis and the platelet count was 158 x 10^9/l
By 4 hours phagocytosis was extensive
Angiogram was uneventful
Laboratory Errors leading to Inappropriate Platelet Transfusion

**2007 SHOT report**
- Platelet aggregation* (2 cases)
- Erroneous POCT (1 case)

* In one case despite a comment that there was aggregation and a request for a citrated sample

Verification of the Platelet Count

- Platelet counts that are unexpectedly normal should also be verified
- All platelet counts in patients with acute leukaemia with a high blast cell count should be verified, whether low or normal
- Platelets may be simulated by red cell fragments, micro-organisms, cryoglobulin, fragments of leukaemic cells

Micro-organisms Simulating Platelets

- A 50-year-old woman had received chemotherapy for AML
- Having been profoundly thrombocytopenic and platelet dependent for 10 weeks, her platelet count (Technicon H2) showed a sudden improvement from 27 to 56 x 10^9/l

Blood film

* Image from Bain *Blood Cells* 
Blackwell Science 2002
Micro-organisms Simulating Platelets

- Blood culture (from a vein and from an indwelling line) grew *Candida glabrata*
- She was given AmBisone and the line was removed
- Her ‘platelet count’ fell to 6 x 10⁹/l

White Cell Fragments Simulating Platelets

- Occasional seen in lymphoma and hairy cell leukaemia
- Common, although rarely recognized, in AML and ALL

White Cell Fragments Simulating Platelets

- Blood films of 169 patients with AML or ALL were reviewed
- A 500-particle count was performed
- More than 5% of ‘pseudoplatelets’ as a proportion of platelets plus pseudoplatelets was regarded as significant and recorded

Van der Meer et al. 2003 *J Clin Pathol*, 56, 772

White Cell Fragments Simulating Platelets

- 30% of films from patients with AML and 18% of films from patients with ALL had more than 5% of pseudoplatelets
- Eleven of 43 patients with increased pseudoplatelets were thought, retrospectively, to have been at risk of haemorrhage as the count was < 15 x 10⁹/l

Van der Meer et al. 2003 *J Clin Pathol*, 56, 772
White Cell Fragments Simulating Platelets

- Their automated counts (H3) ranged from 10 to 75 x 10^9/l
- Their corrected counts ranged from 2 to 15 x 10^9/l

Van der Meer et al. 2003 J Clin Pathol, 56, 772

Verification of the Platelet Count

- Unexpectedly high platelet counts should also be verified
- Platelets may be simulated by red cell fragments, micro-organisms, cryoglobulin, fragments of leukaemic cells
- If the platelet count is true the film may give a clue as to the reason

“Thrombocytosis”

- Is it genuine or artefactual?
Thrombocytosis

- We have already seen a factitious platelet count of 1539 x 10^9/l resulting from accidental heating of a blood sample.

- The same thing can happen in thermal burns.
- This patient had a 'platelet' count of 748 x 10^9/l.
  Sapanara et al. 2004 Lab Med. 35, 98.

- The same phenomenon may be seen in red cell fragmentation syndromes.
- This is ciclosporin-induce MAHA with many platelet sized schistocytes.

- More a problem with impedance counters than light scattering instruments.
- Platelets have a different refractive index from red cells.
A Platelet Count of $2300 \times 10^9/l$

- Routine platelet count $2300 \times 10^9/l$
- Platelet count done immediately after taking blood $162 \times 10^9/l$
- **What is the problem?**

*Clin Lab Haematol, 25, 1-8, 2003.*

With thanks to Dr. Guy Lucas, Manchester.
A Platelet Count of 2300 x 10^9/l

So what is it?

- High power

Factitious Hb, Red Cell Indices or Both

- Turbidity from leucocytosis
- Turbidity from hyperlipidaemia
- Cold agglutinins
- Cryoglobulins

White Cell Count is Too Low

- Agglutination

Morphology Can Tell You Things That No Automated Instrument Can Yet Tell You

- The cause of thrombocytopenia
- The cause of anaemia, particularly but not only haemolytic anaemias and macrocytic anaemias
- Likely myelodysplastic syndromes
- The likely nature of various types of leukaemia and lymphoma
- That there are bacteria, fungi or parasites
The Cause of Thrombocytopenia

- A 68-year-old Kashmiri man
- Chest pain and dyspnoea for 2 months
- Past medical history
  - Hypertension
  - Diabetes mellitus
  - Chronic obstructive pulmonary disease
  - Hepatitis C infection (on peginterferon and ribavarin)

- The patient developed respiratory failure
- Renal function deteriorated
- Why has the platelet count fallen

Observation of the blood film led to a diagnosis of interferon-induced microangiopathy


The Cause of Anaemia

Why is he jaundiced?

The Cause of Anaemia

Why has this patient with Burkitt lymphoma suddenly become anaemic? (Image 1)


The Cause of Anaemia

Why has this patient with Burkitt lymphoma has suddenly become anaemic? (Image 2)


The Cause of Anaemia

Why does this anaemic young woman have liver failure?


The Cause of Anaemia

• It is fairly obvious why this patient has anaemia

The Cause of Anaemia

• But this is more subtle. Why is this patient anaemic?

A Blood Film May Show Something that no Automated Instrument can yet Reveal

A Pregnant Nigerian Woman

• A pregnant Nigerian woman arrived in the UK at 37 weeks gestation in her first pregnancy
• She saw her GP complaining of tiredness and shortness of breath for 3 weeks
• She denied any other symptoms
• A blood count was done

A Pregnant Nigerian Woman

• TNCC 112.8 x 10⁹/l
• RBC 1.72 x 10¹²/l
• Hb 55 g/l
• Hct 0.17 l/l
• MCV 101 fl
• MCH 31.7 pg
• MCHC 315 g/l
• Platelet count 471 x 10⁹/l
A Pregnant Nigerian Woman

- What would a haematologist do next?
A Pregnant Nigerian Woman

- What is the underlying diagnosis?
- Why is the patient so severely anaemic?

- Her HPLC looked like this – haemoglobins S, F and A₂ only

Some causes of more severe anaemia in sickle cell anaemia
- Splenic sequestration (infants)
- Parvovirus B19 infection
- Other infection
- Megaloblastic anaemia
- ? Anything else

- Manual NRBC count 102 x 10⁹/l
- Corrected WBC 10.6 x 10⁹/l
- Reticulocyte count increased
- Note also MCV was 101 fl
- No neutrophil hypersegmentation
- No neutrophil toxic changes
• On further questioning the patient stated she had sickle cell trait
• She thought that she might have had malaria 3 weeks earlier and had taken a single Fansidar tablet and some paracetamol
A Pregnant Nigerian Woman

• What general lessons can we learn
  – Sickle cell disease can be diagnosed from a blood film
  – A presumptive diagnosis of sickle cell anaemia can be made from a blood film
  – Examination of a blood film can show why a patient with sickle cell anaemia has become more anaemic
  – What else can we learn?

One swallow does not make a summer (?)

Sometimes one swallow does make a summer

A Man With Blurred Vision

• He was 34-years-old and presented with blurred vision of 3-weeks duration
• Ophthalmic examination showed bilateral retinal haemorrhages
• A blood count was therefore performed
A Man With Blurred Vision

The FBC showed
- WBC $5.1 \times 10^9/l$, Hb 5.5 g/dl, MCV 103 platelet count $17 \times 10^9/l$
- What is your differential diagnosis at this stage?

By chance, the blood sample was put through two different instruments of the same model, providing a choice of differential count
- One FBC had a normal differential count was normal with no flags
- The other had an abnormal differential count and was flagged

- Neuts 3.0, lymph 1.9, mono 0.1
- No flags — a normal differential with no flags

- Neuts 0.5, lymph 2.1, mono 2.5
- Flags ‘No blasts, verify diff’— an abnormal differential with a flag
• To the human eye, the plots look quite similar

A Man With Blurred Vision

A Man With Blurred Vision
• The patient was contacted on a Saturday evening and asked to come to the hospital
• The on-call haematologist found him to have massive splenomegaly and a groin rash, suggestive of a fungal infection
• What is your differential diagnosis now?
• Perhaps a blood film will help

Blood film

Blood film
A Man With Blurred Vision

Blood film

A Man With Blurred Vision

Blood film

A Man With Blurred Vision — Immunophenotype

CD19  99%  CD5  neg
FMC7  96%  CD2  neg
CD79b 97%  CD23  neg
CD22  99%  CD25  99%
CD11c 99%  CD103  78%
CD123 98%
Lambda 92%  Kappa negative

Not Only —— But also

www.trinityhigh.co.uk/tales/goodbye.htm
Not Only May a Blood Film Show Something that no Automated Instrument can Yet Reveal

But Also it Can Permit a Very Rapid Provisional Diagnosis in Haematological Emergencies
A Haematological Emergency

• But what about this case?

Is This a Haematological Emergency?

• ..... and this case?

Is this a Haematological Emergency?

• ... and another case

Are Blood Films Misinterpreted?

• Maybe......
A NEQAS Exercise

An HIV-positive man with caecal Burkitt lymphoma

A NEQAS Exercise

An HIV-positive man with caecal Burkitt lymphoma

A NEQAS Exercise

• Many participants made a misidentification of spherocytes which led to 20% or 503 participants suggesting HS or AIHA
• However a third of participants identified irregularly contracted cells, ghost cells, hemi-ghosts, 'blister cells' or 'bite cells' (actually quite infrequent)
• This often led to the correct suggestion of G6PD deficiency

A NEQAS Exercise

• The patient had a normal G6PD assay at this stage
• He had the African type of G6PD deficiency
• Detecting the morphological evidence of oxidant damage is thus very important
• He had been prescribed rasburicase
Another NEQAS Exercise

- A blood film was provided with the following details:
  - 76-year-old man
  - Generally unwell
  - WBC 4 x 10⁹/l, Hb 57 g/l, platelet count 56 x 10⁹/l

Another NEQAS Exercise

- A third of laboratories did not report macrocytes
- Red cell fragments were noted
- 12% of laboratories mistakenly suggested a diagnosis of MAHA or TTP
- Serum B₁₂ was 30 ng/l (NR 160-900)
- The patient had pernicious anaemia
Diagnosis requires integration of information from all sources.

What happens if we omit blood film morphology?

Diagnosis may fail.

Conclusion

We Can Still Learn from the Blood Film