The role of blood film morphology in thrombocytopenia, thrombocytosis and other platelet disorders

Barbara J Bain

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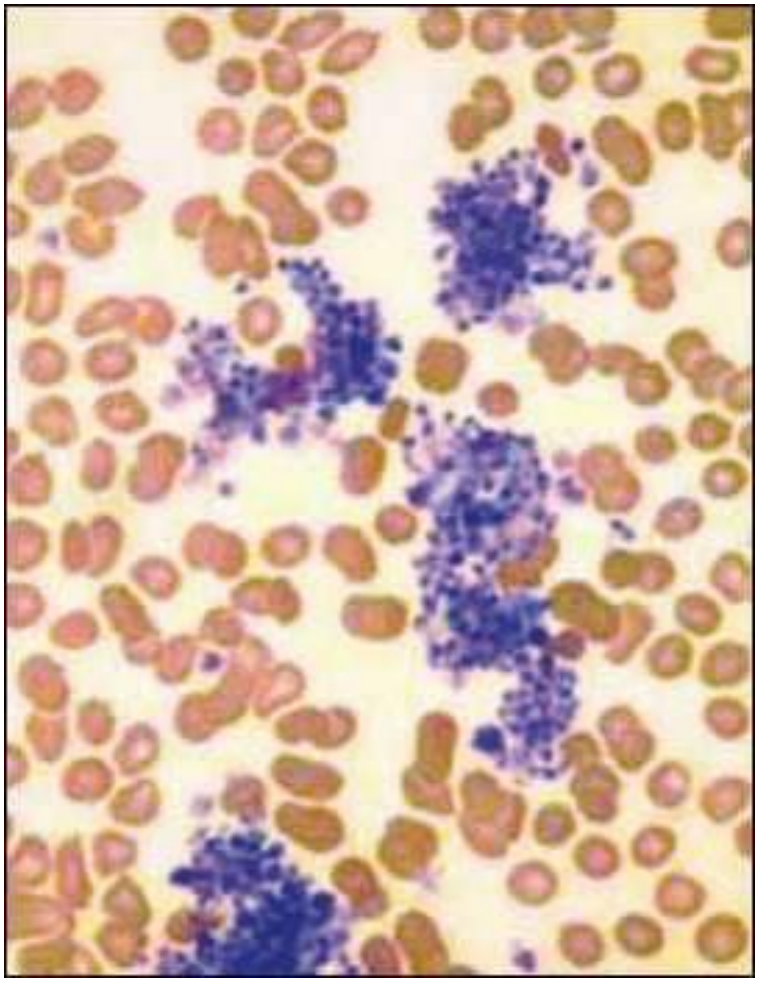
Thrombocytopenia – why examine the blood film?

- Validation of the count
- Explanation of abnormal platelet scatter plots
- Identification of the cause of thrombocytopenia
- Avoidance of unnecessary or contraindicated platelet transfusions

Validation of a low platelet count

Platelet aggregation

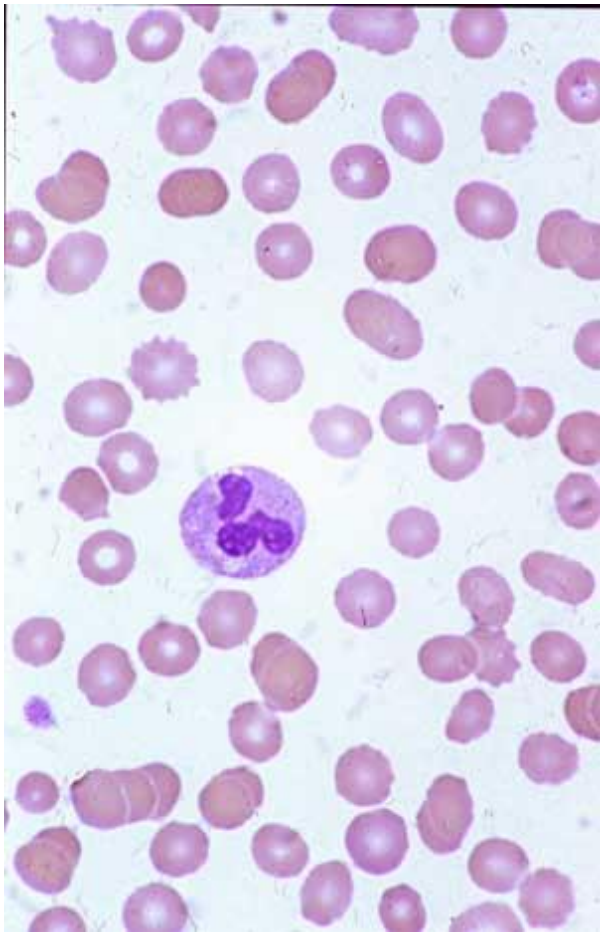


Bain BJ (2005) Diagnosis from the blood smear. *N Engl J Med*, **353**, 498-507.

Validation of a low platelet count

Platelet aggregation

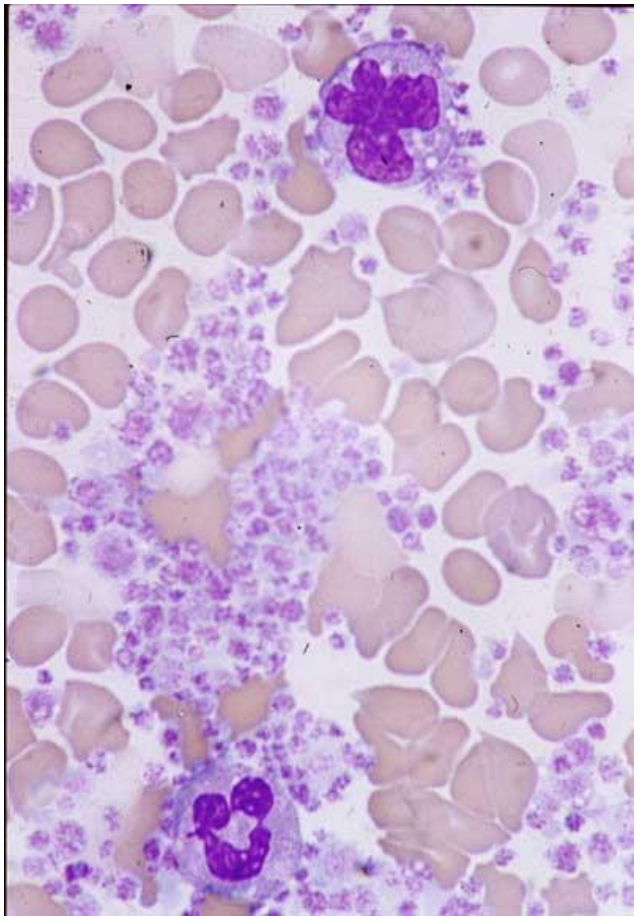
The body of the film – the count appears genuine



Validation of a low platelet count

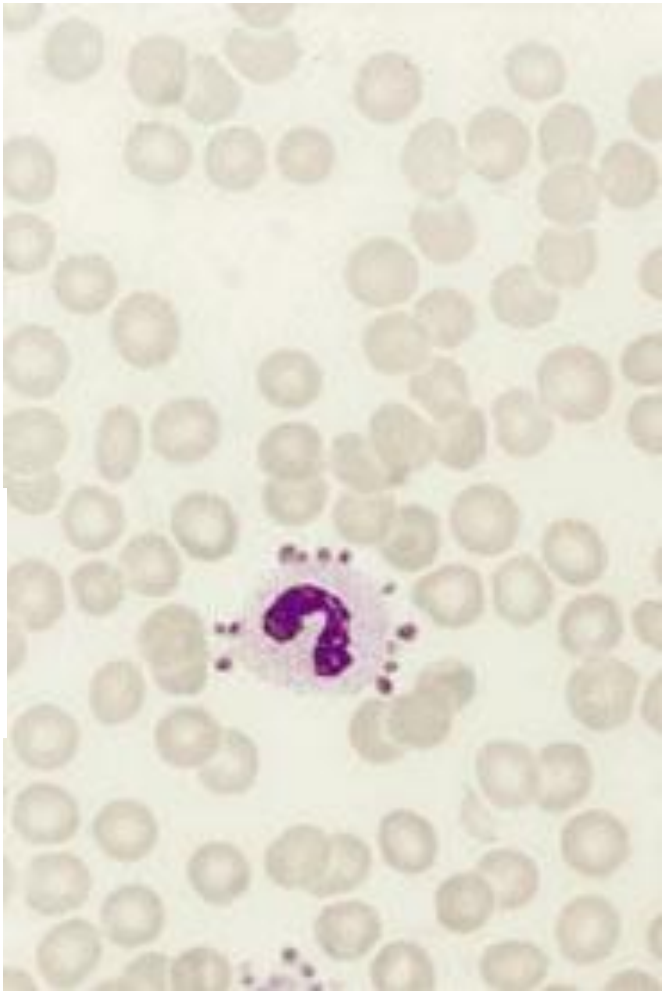
Platelet aggregation

The tail of the film – the artefact is revealed



Validation of a low platelet count

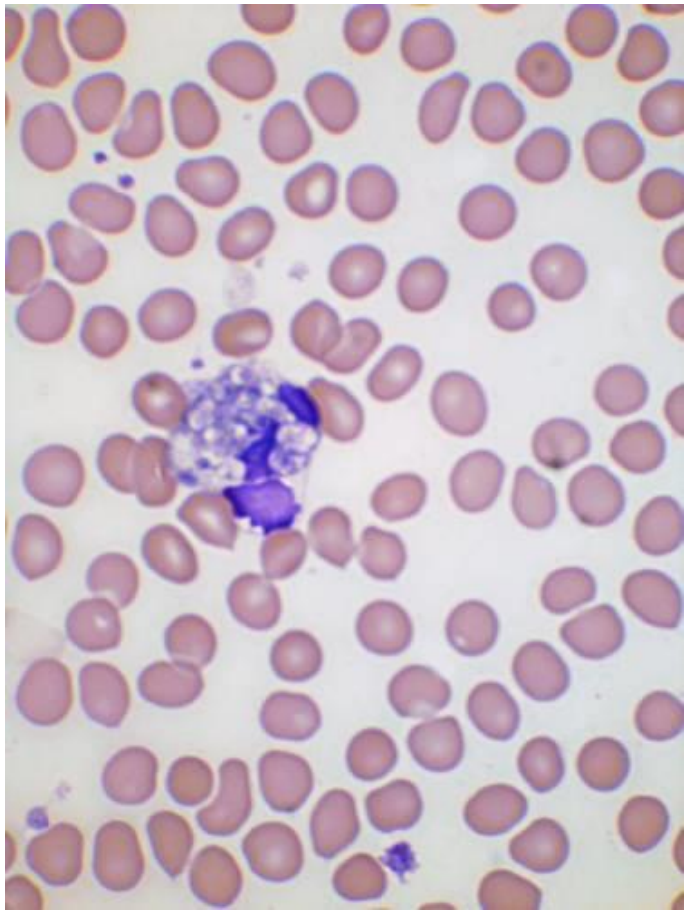
Platelet satellitism



Bain BJ (2015) Blood Cells, 5th edn. *Wiley-Blackwell, Oxford*.

Validation of a low platelet count

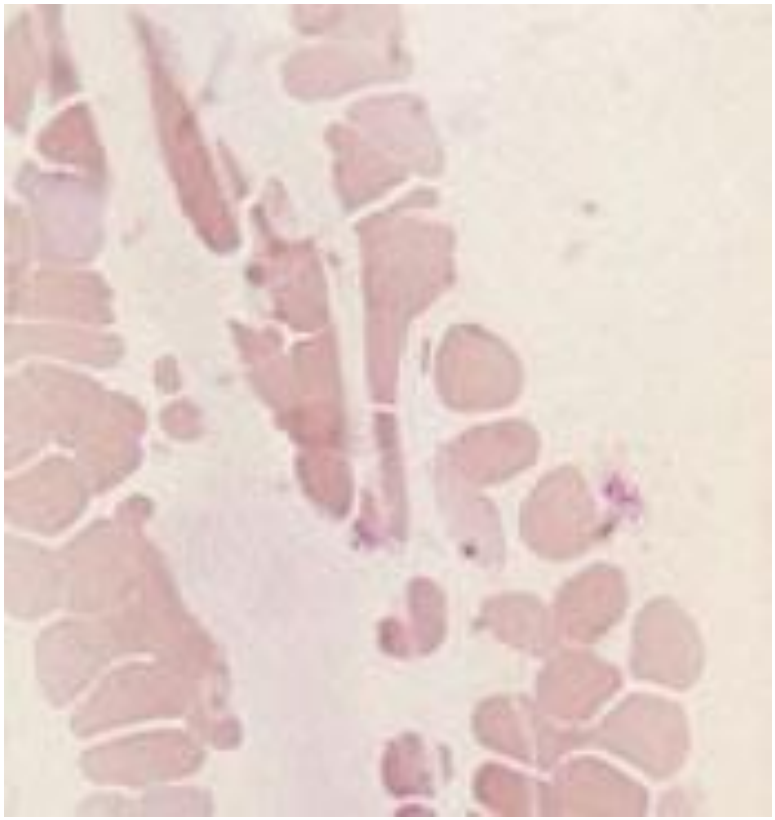
Platelet satellitism followed by platelet phagocytosis



Campbell V, Fosbury E and Bain BJ (2009) Platelet phagocytosis as a cause of pseudothrombocytopenia. *Am J Hematol*, **84**, 362.

Validation of a low platelet count

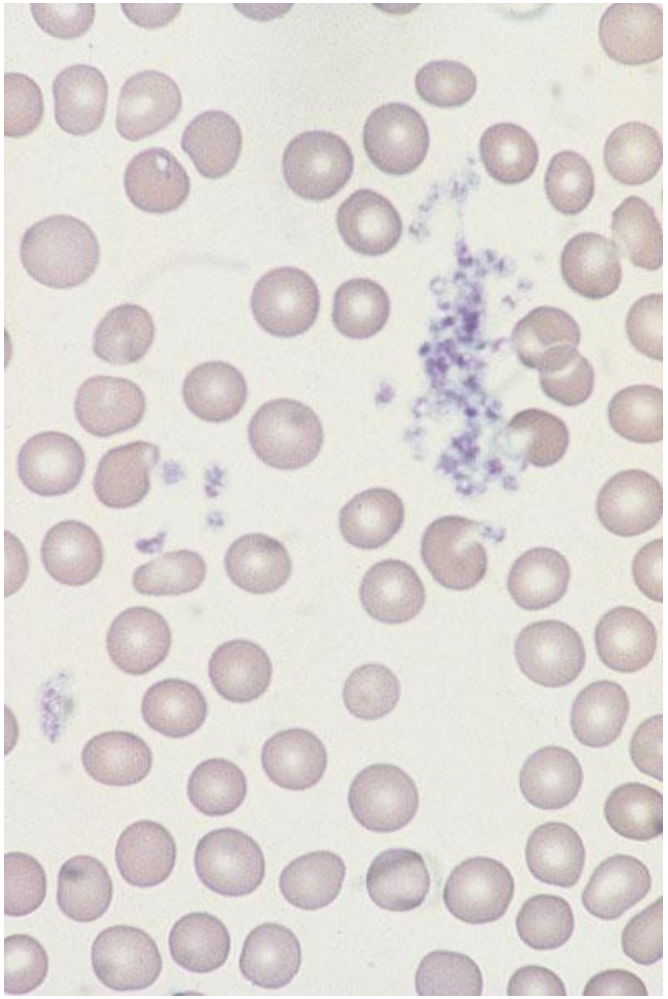
Fibrin strands – likely to indicate a difficult venepuncture or poorly mixed specimen



Bain BJ (2015) Blood Cells, 5th edn. *Wiley-Blackwell, Oxford*.

Validation of a low platelet count

Platelet aggregation



Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley-Blackwell, Oxford.

Explanation of abnormal platelet indices, histograms and scatter plots

- A film can be informative when there is an increased MPV, increased PDW and abnormal scatter plots
- The explanation may be the grey platelet syndrome or a pseudo-grey platelet syndrome
- The platelet count should also be validated when there is marked microcytosis

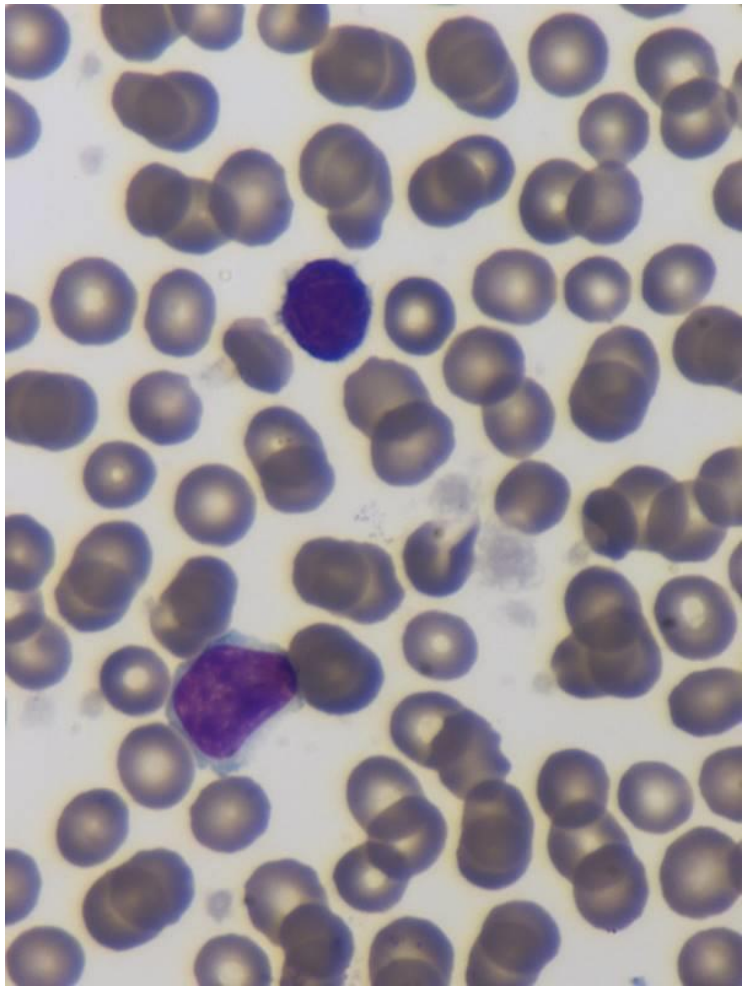
A complex case explained

- A 6-year-old boy with phenylketonuria was being treated with a drug known to cause thrombocytopenia
- An FBC showed a normal platelet count of 167 × 10^9/l but scatter plots suggested agranular platelets
- There was no history of abnormal bleeding

Pancione Y, Fumi M, Sale S, Rocco V and Bain BJ (2016) Gray platelets - artifact or real? *Am J Hematol*, **91**, 538.

A complex case explained

- This is his blood film (EDTA-anticoagulated)
- Is this the grey platelet syndrome or pseudo-grey platelet syndrome?



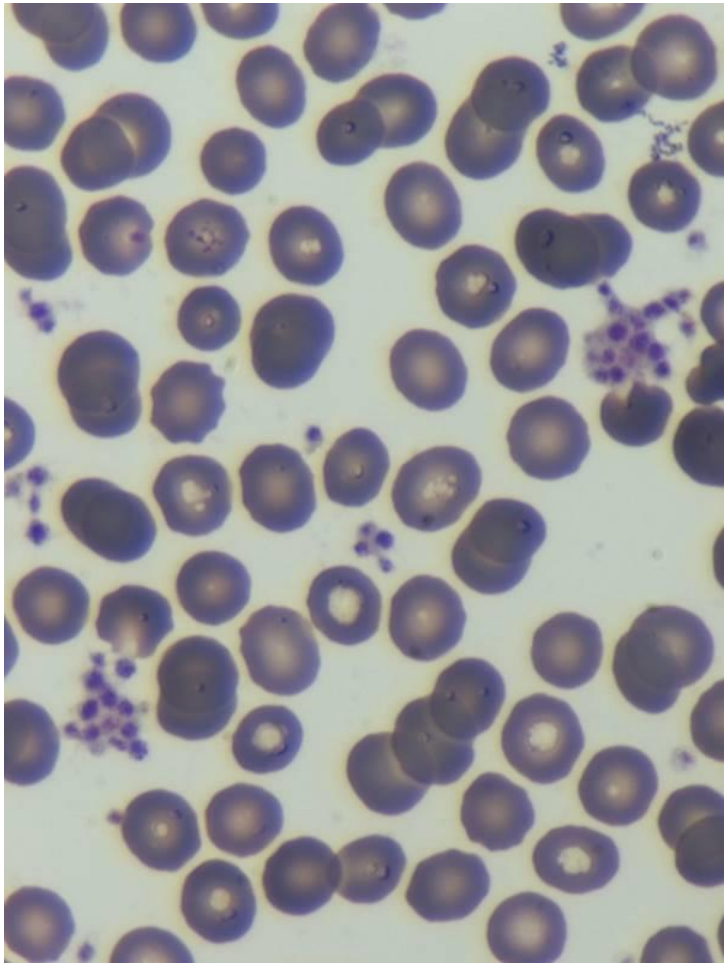
A complex case explained

Is this the grey platelet syndrome or pseudo-grey platelet syndrome?

- A finger-prick film showed normal platelet numbers and morphology

A complex case explained

- However a citrated sample showed ‘thrombocytopenia’
- Platelets were normal in number, size and granularity

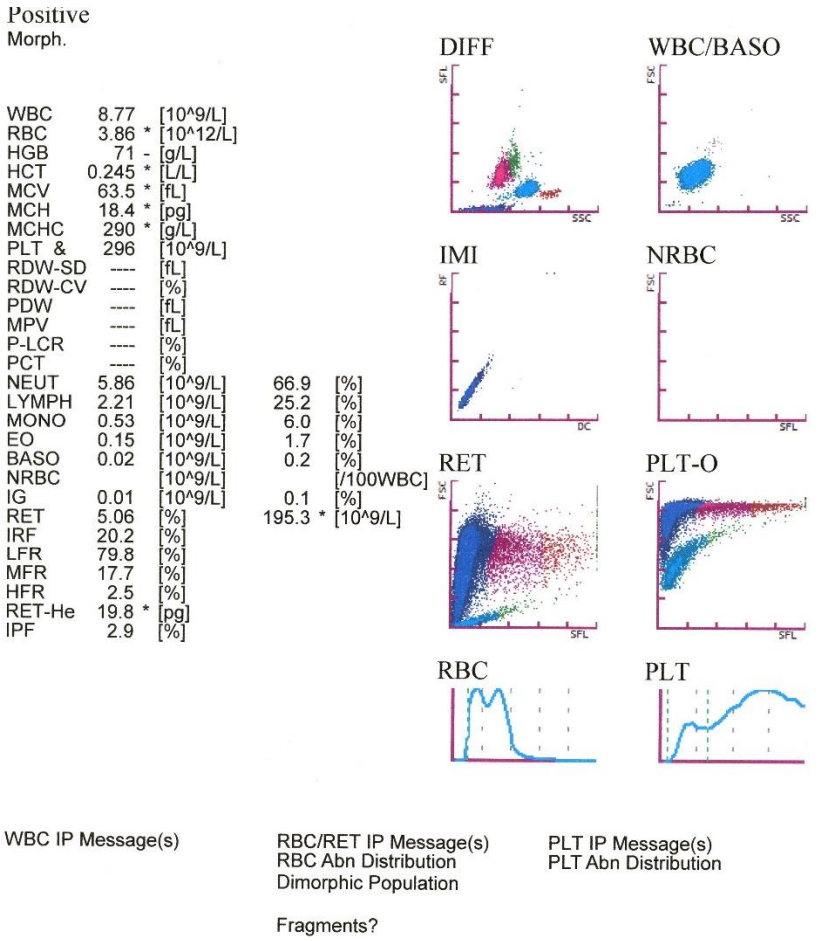


A complex case explained

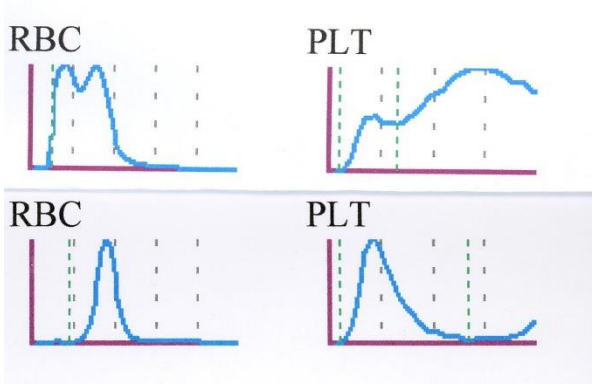
- The patient thus had two *in vitro* artefacts
- The pseudo-grey platelet syndrome is due to an antibody that stimulates release of α and δ granules after chelation of Ca by EDTA
- The platelet aggregation is another *in vitro* antibody-mediated phenomenon, more common with EDTA-anticoagulated blood

Explanation of abnormal platelet indices, histograms and scatter plots

- This patient was mistakenly treated for ‘iron deficiency anaemia’



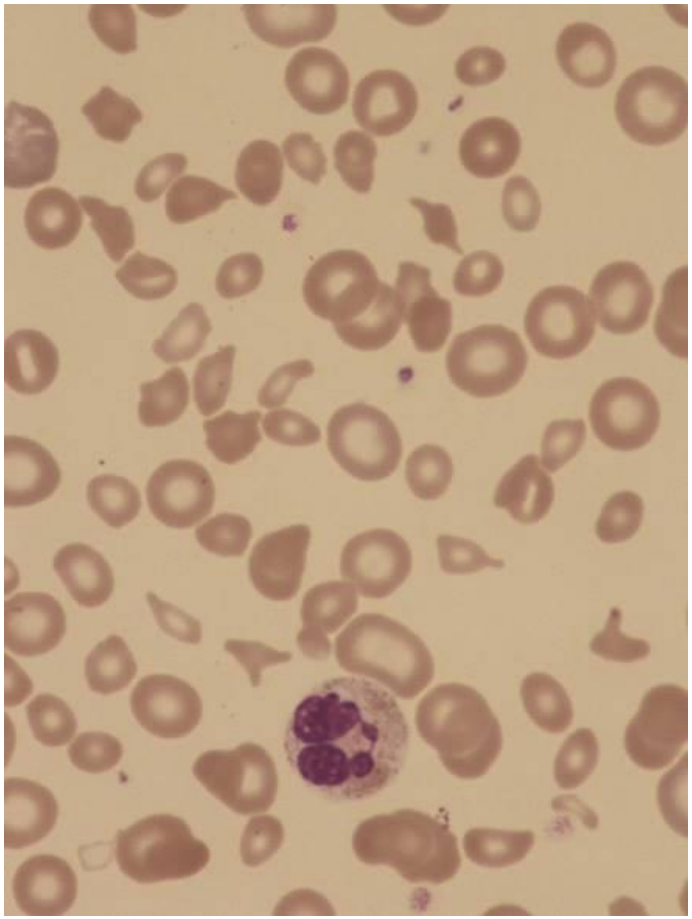
Explanation of abnormal platelet indices, histograms and scatter plots



Patient

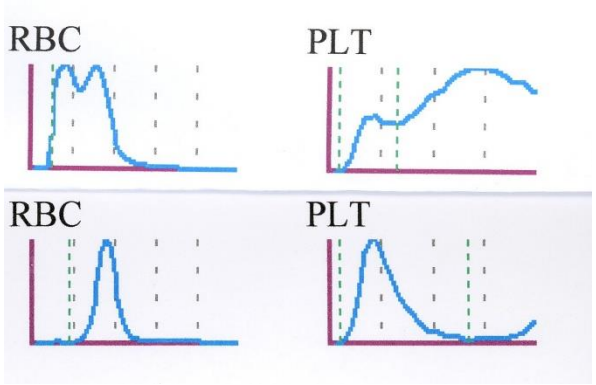
Normal control

- What do the blood film and histograms tell us?



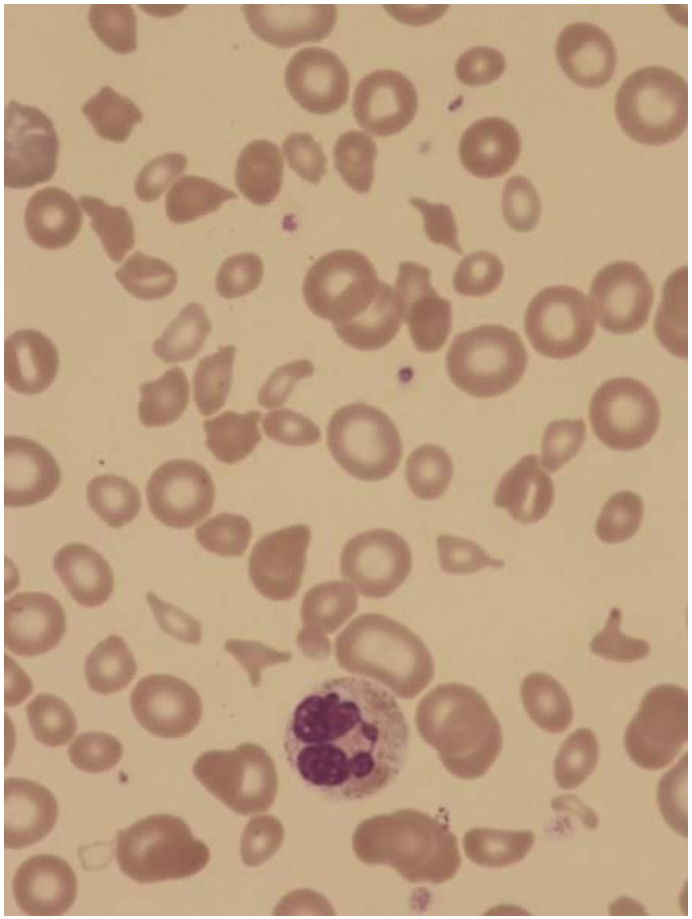
Bain BJ, Varu V, Rowley M and Foale R (2015) Mechanical hemolysis: a low mean cell volume does not always represent microcytosis. *Am J Hematol*, **90**, 1179.

Explanation of abnormal platelet indices, histograms and scatter plots



Patient

Normal control



- Platelet count is likely to be wrong
- Low MCV is not due to microcytosis

Identification of the cause of thrombocytopenia

- Is it an inherited condition?
  - Are platelets small, normal sized or large
  - Are platelets agranular or hypogranular?
- Is it acquired?
  - Are there blast cells or leukaemic promyelocytes?
  - Are there organisms?
  - Are there schistocytes?
  - Are there any other clues?

A few of the inherited causes of thrombocytopenia

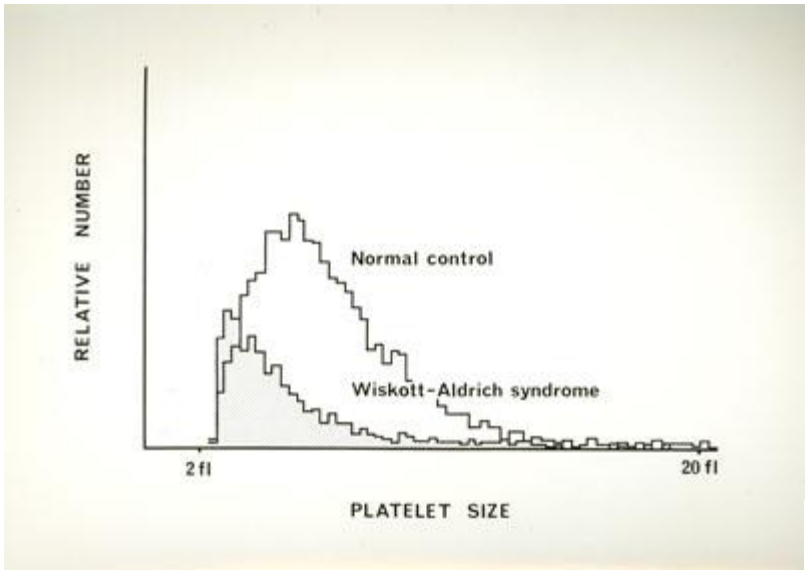
- Small platelets
  - Wiskott–Aldrich syndrome
- Normal sized platelets
  - Thrombocytopenia with absent radii
  - Amegakaryocytic thrombocytopenia with radio-ulnar synostosis
  - Fanconi anaemia
  - Familial platelet disorder with a propensity to AML
  - … and many others

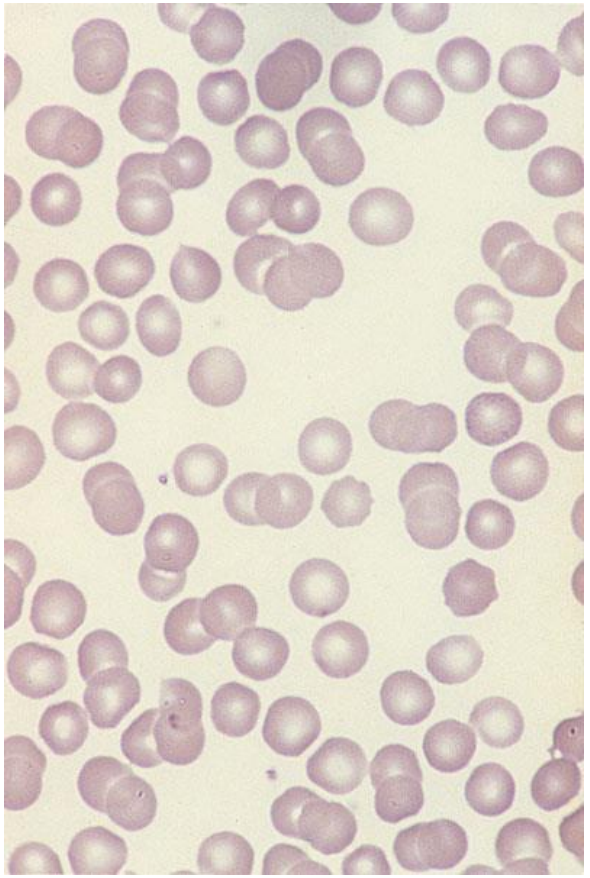
A few of the inherited causes of thrombocytopenia

- Large platelets
  - Bernard–Soulier syndrome
  - *MYH9*-related disorders
  - Grey platelet syndrome
  - Di George syndrome
  - Phytosterolaemia
  - … and many others

Wiskott–Aldrich syndrome

- Small platelets

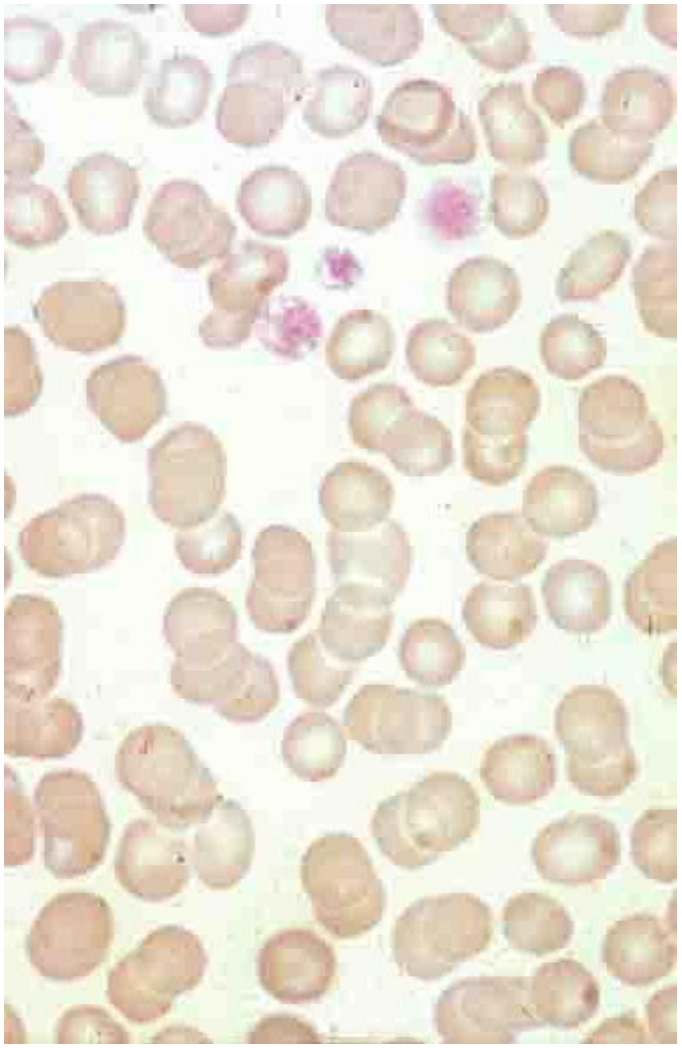




Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley-Blackwell, Oxford.

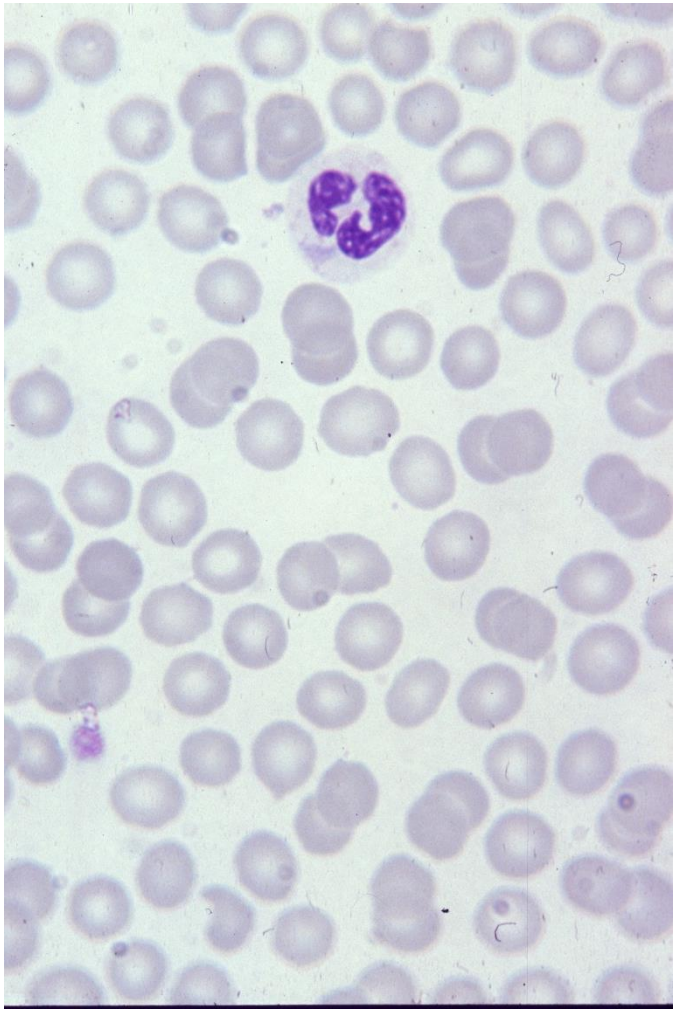
Bernard–Soulier syndrome

- Large platelets, normally granulated



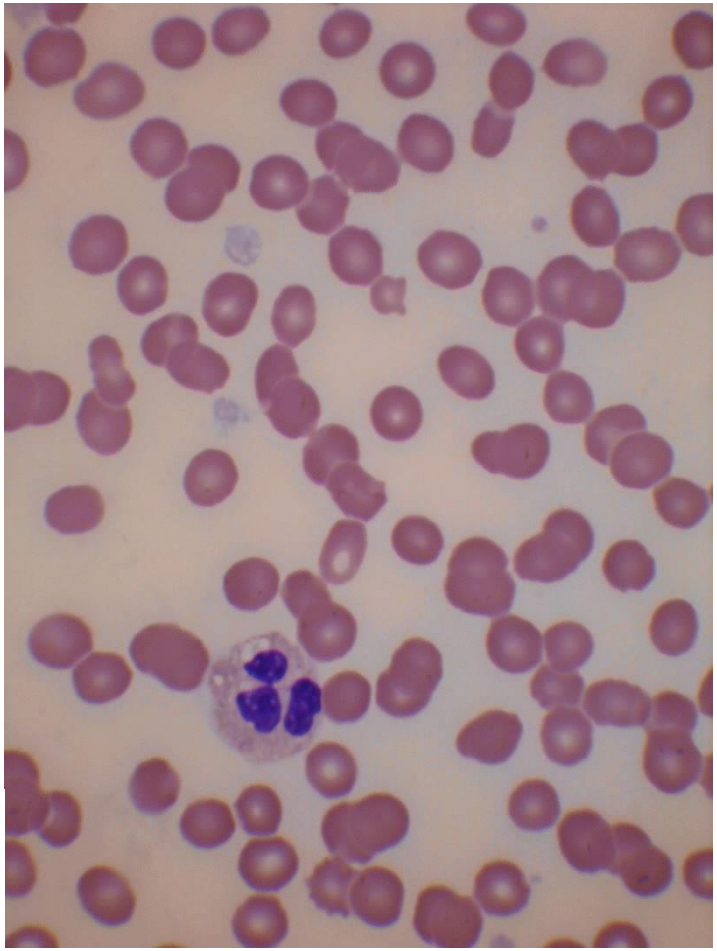
MYH9-related disorders

- May–Hegglin and related anomalies
- Granulocyte inclusions (Döhle-like bodies)
- NOTE: inclusions are sometimes absent and can be difficult to see



Grey platelet syndrome

- A 20-year-old man with significant haemorrhage into his thigh following a sporting injury
- Platelet count 120 × 10^9/l



Bain BJ and Bhavnani M (2011) The gray platelet syndrome. *Am J Hematol*, **86**, 1027.
Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley–Blackwell, Oxford

An unusual but important cause of (thrombocytopenia with) large platelets

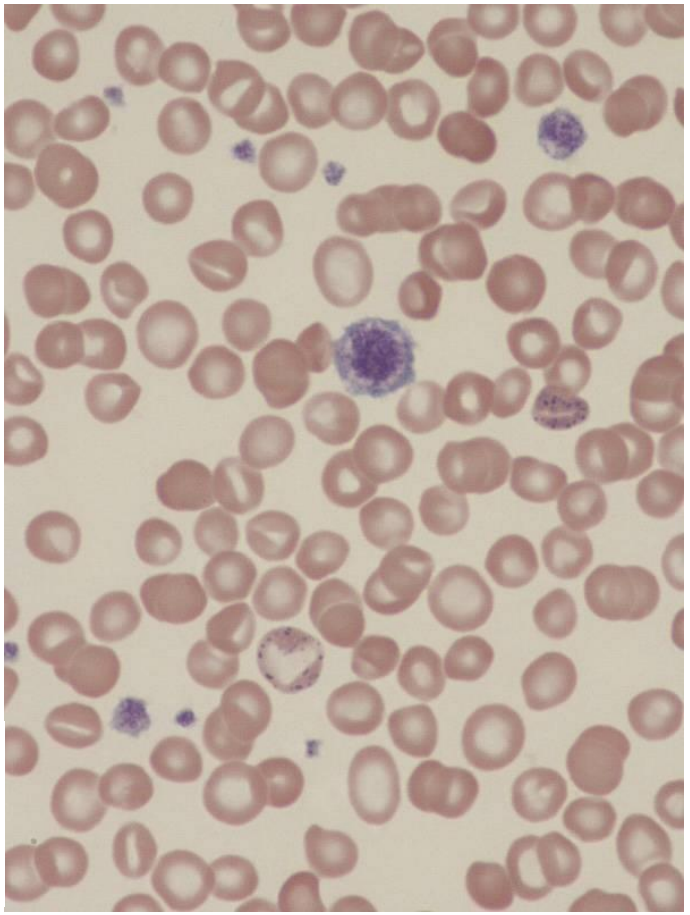
- A 12-year-old Iranian boy
- Parents first cousins
- Known β thalassaemia trait
- Anaemia refractory to iron therapy
  − features of anaemia of chronic disease: low iron, transferrin and transferrin saturation with serum ferritin 375 μmol/ml (15–300)

An unusual but important cause of (thrombocytopenia with) large platelets

- Hb 88 g/l, MCV 56.8 fl, MCH 17.6 pg, MCHC 309 g/l, platelet count 209 × 10^9/l, reticulocytes 156 and 191 × 10^9/l
- Lactate dehydrogenase 249 iu/l (200–450)

An unusual but important cause of (thrombocytopenia with) large platelets

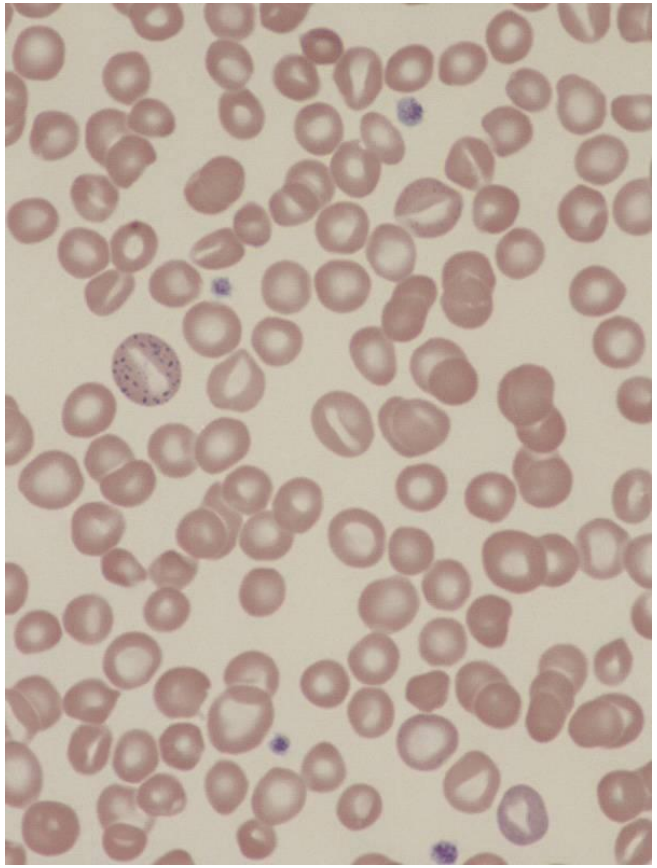
- The blood film, in addition to the features of β thalassaemia trait, showed giant platelets and stomatocytosis
- What is the diagnosis and does it matter?



An unusual but important cause of (thrombocytopenia with) large platelets

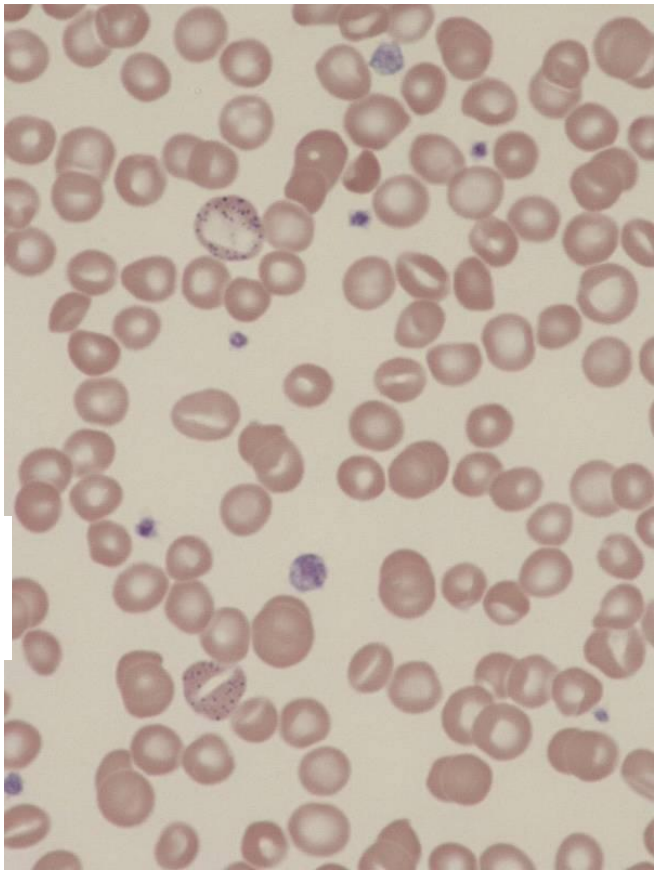
What is the diagnosis?

- Phytosterolaemia (also known as sitosterolaemia)
- An AR condition (mutated *ABCG5* or *ABCG8* gene) with increased absorption of plant and other sterols



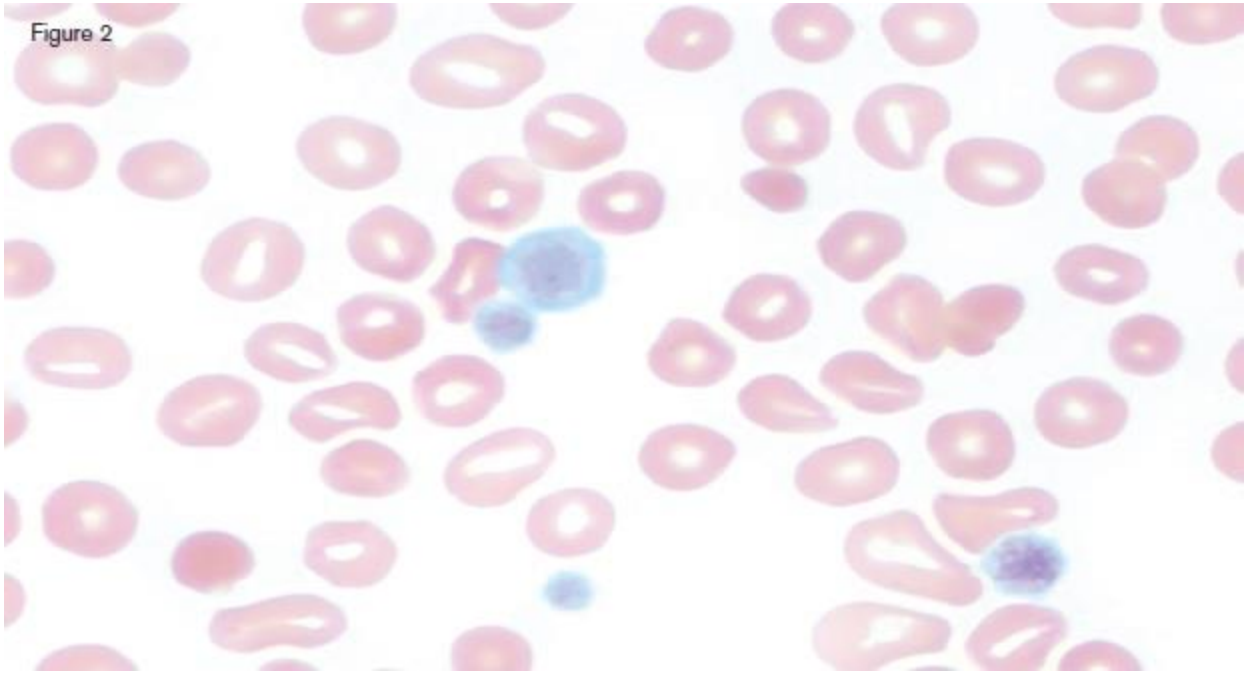
An unusual but important cause of (thrombocytopenia with) large platelets

- It matters because it causes premature vascular disease and there is now a specific treatment, ezetimibe, a sterol pump inhibitor
- Diagnosis in the child led to diagnosis also in his brother



Bain BJ and Chakravorty S (2016) Phytosterolemia, *Am J Hematol,* **91**, 643.

Another example of phytosterolaemia

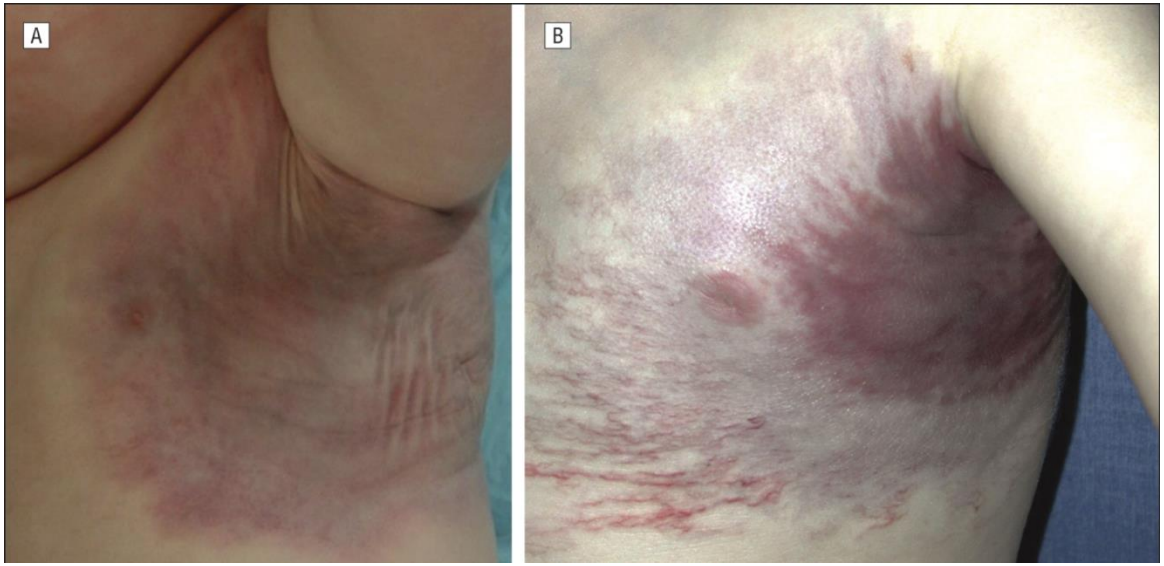


Dietrich B and Berube C (2017) Planting evidence. *The Hematologist* http://www.hematology.org/Thehematologist/Features/7130.aspx

Another constitution abnormality indirectly causing thrombocytopenia

- A congenital vascular malformation
- Platelet count

  $17 \times 10^9/l$



Age 4 months © 2 and a half years

Alomari (2010) *Arch Pediatr Adolesc Med,* 164, 387.

So what do you do if you suspect congenital thrombocytopenia?

- Clinical assessment – is it syndromic?
- Automated count including MPV, PDW, IPF
- Blood film
- Platelet aggregometry (might include Lumi aggregometry)
- Next generation sequencing panel of ~ 80 genes: ThromboGenomics, Addenbrooke's

Thanks to Andrew Mumford, Bristol

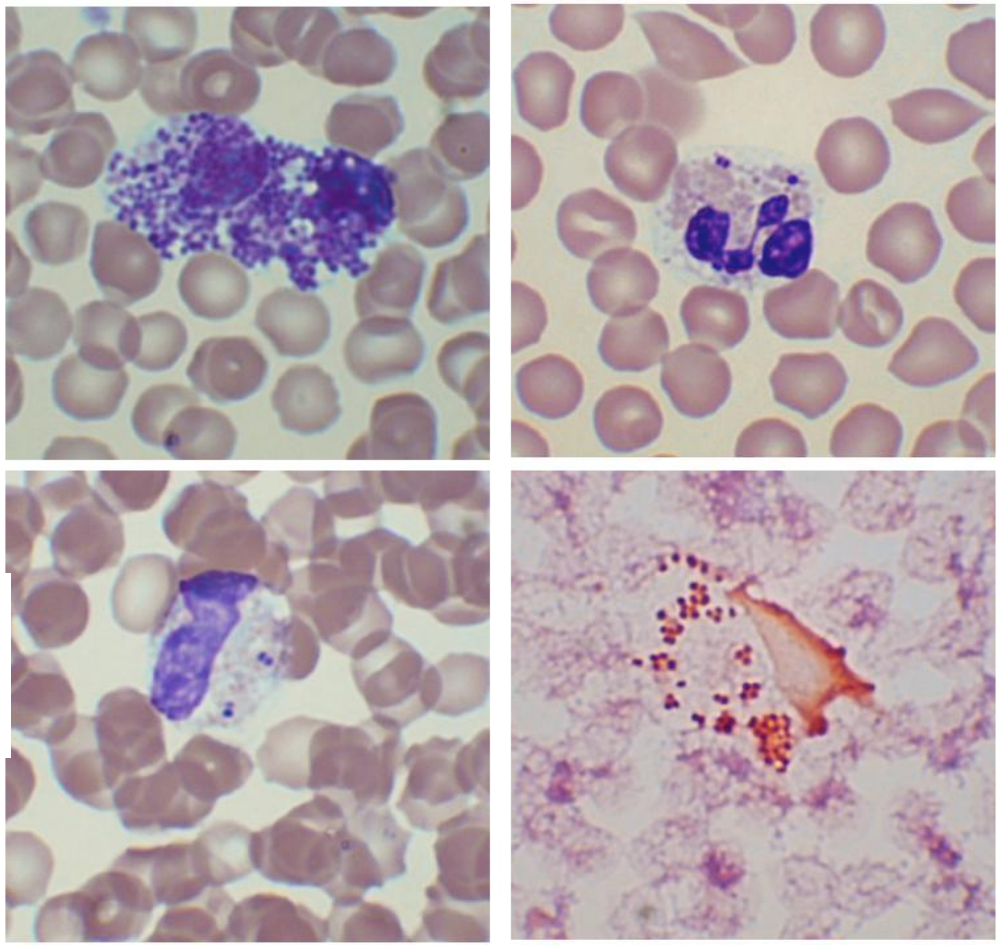
http://thrombo.cambridgednadiagnosis.org.uk/

Some acquired causes of thrombocytopenia – are there organisms?

- A 29-year-old woman was admitted to Accident and Emergency
- An FBC showed: Hb152 g/l, WBC 9.6 × 10^9/l and platelet count 39 × 10^9/l
- A citrate sample for coagulation tests was unclottable
- APL was suspected

Some acquired causes of thrombocytopenia – are there organisms?

- What is the diagnosis?



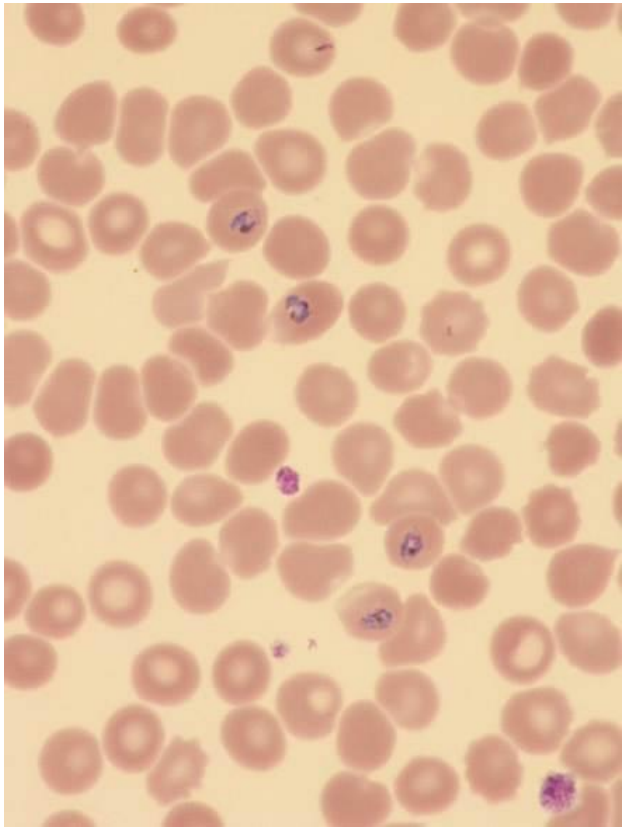
Uprichard J and Bain BJ (2008) A young woman with sudden onset of a severe coagulation abnormality. *Am J Hematol*, **83**, 672.

Some acquired causes of thrombocytopenia – can we have some platelets please?

- A 63-year-old man presented to a London hospital on a Saturday morning with abdominal pain, fever, diarrhoea and vomiting after a buffet meal
- FBC showed; WBC 5.1 × 10^9/l, Hb 148 g/l and platelet count 21 × 10^9/l
- Over the weekend he developed hypotension, tachycardia and deteriorating mental state

Some acquired causes of thrombocytopenia – can we have some platelets please?

- On Monday morning the haematology department were asked for platelets to cover a femoral line insertion
- What is the diagnosis?



Some acquired causes of thrombocytopenia – can we have some platelets please?

- What is the diagnosis?
- Falciparum malaria
- The patient was Somalian with a presumptive diagnosis of gastroenteritis and no travel history had been taken
- The moral of the story – don't put your microscopes on ebay

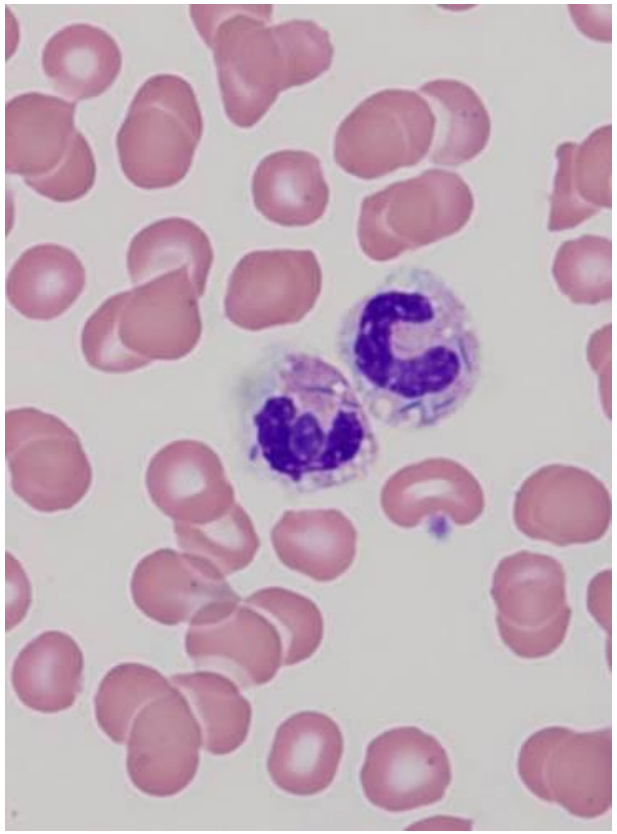
Siow W and Bain BJ (2017) Can we have some platelets please? A reason not to put your microscope on eBay. *Am J Hematol*, **92**, 583.

Some acquired causes of thrombocytopenia – are there organisms?

- A 60-year-old Australian woman presented in septic shock after having been bitten by a dog
- An FBC showed: Hb113 g/l, WBC 11.3 × 10^9/l and platelet count 36 × 10^9/l
- She developed DIC, purpura fulminans and multiorgan failure
- She had had a splenectomy 24 years earlier

Some acquired causes of thrombocytopenia – are there organisms?

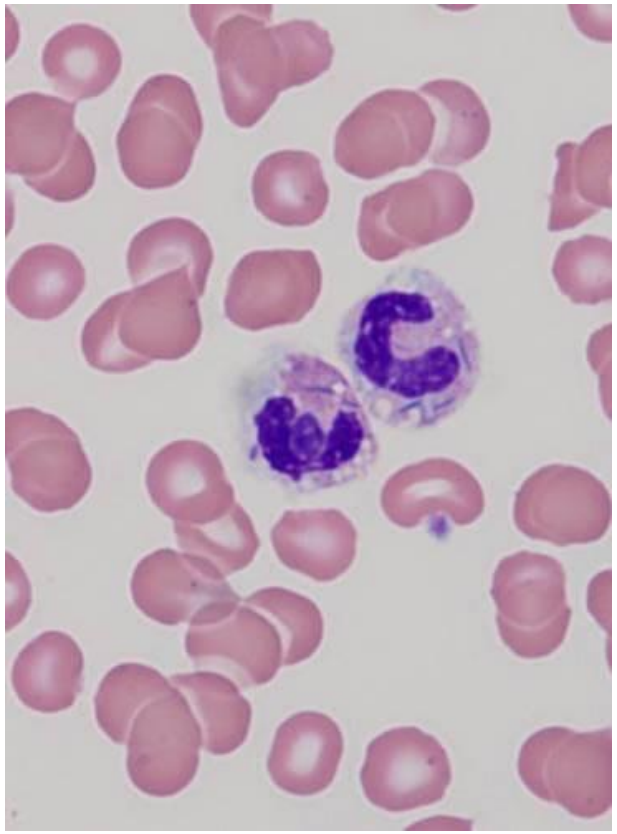
- A 60-year-old hyposplenic woman who had been bitten by a dog
- What is the diagnosis?



Tay HS, Mills A and Bain BJ (2012) Diagnosis from a blood film following dog-bite. *Am J Hematol*, **87,** 915.

Some acquired causes of thrombocytopenia – are there organisms?

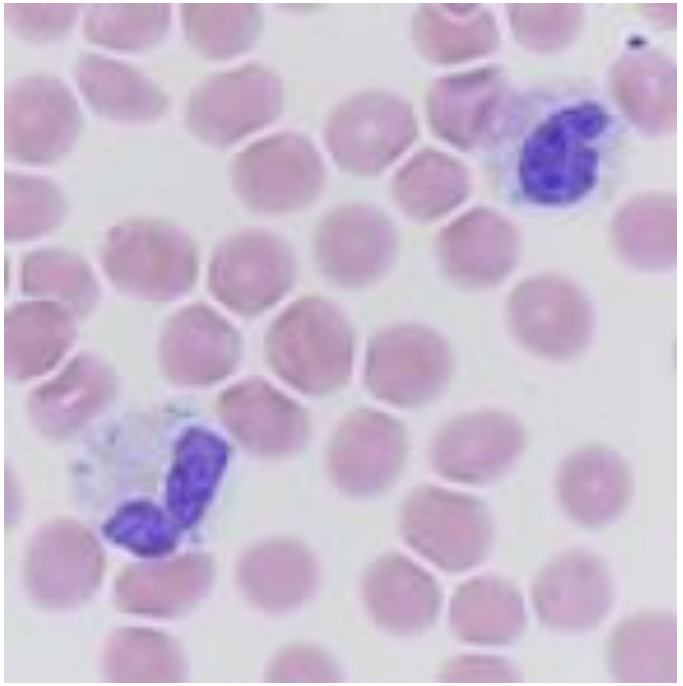
- A 60-year-old hyposplenic woman who had been bitten by a dog
- What is the diagnosis?
- *Capnocytophaga canimorsus*



Tay HS, Mills A and Bain BJ (2012) Diagnosis from a blood film following dog-bite. *Am J Hematol*, **87,** 915.

Some acquired causes of thrombocytopenia – are there organisms?

- *Capnocytophaga canimorsus*
- A similar case has been reported in a dog-walker (who apparently had an intact spleen)



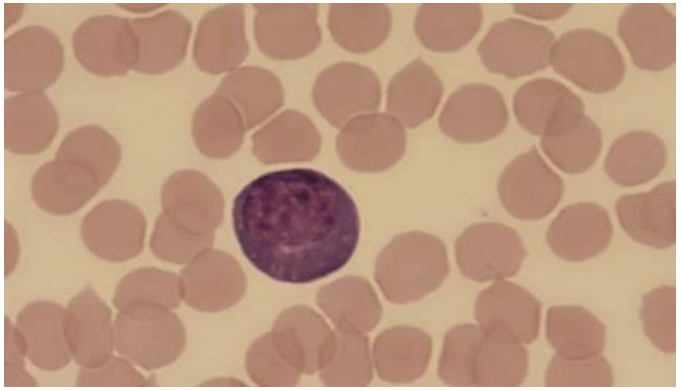
Sokol *et al*. (2017) Atypical hemolytic uremic syndrome associated with *Capnocytophaga canimorsus*. *Am J Hematol*, **92,** 322.

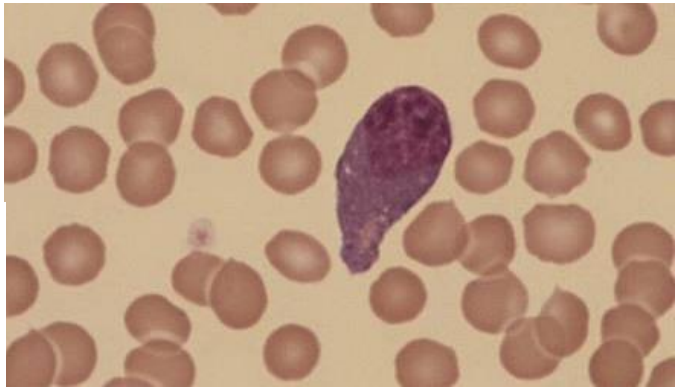
Some acquired causes of thrombocytopenia – is there an organism lurking?

- A 63-year-old febrile man with a Sri Lankan name
- An FBC showed: Hb143 g/l, WBC 4.0 × 10^9/l and platelet count 22 × 10^9/l

Some acquired causes of thrombocytopenia – is there an organism lurking?

- A 63-year-old febrile man with a Sri Lankan name
- What is the diagnosis?

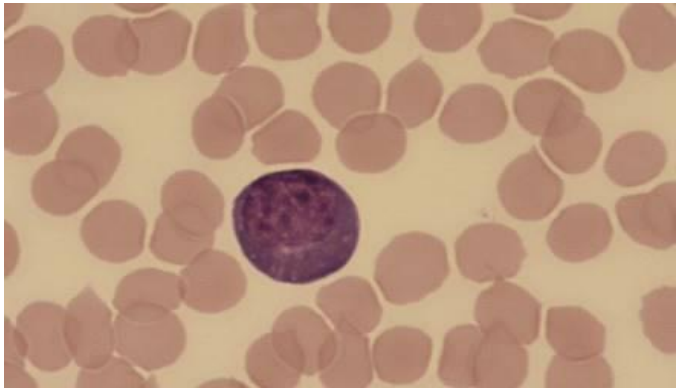


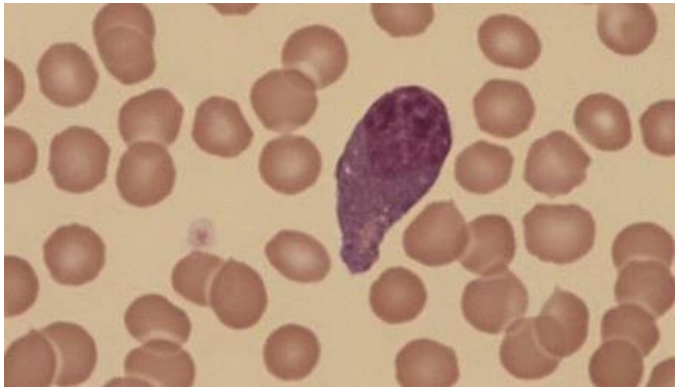


Some acquired causes of thrombocytopenia – is there an organism lurking?

Dengue fever

- Thrombocytopenia
- Atypical lymphocytes without lymphocytosis
- Sometimes leucopenia and neutropenia





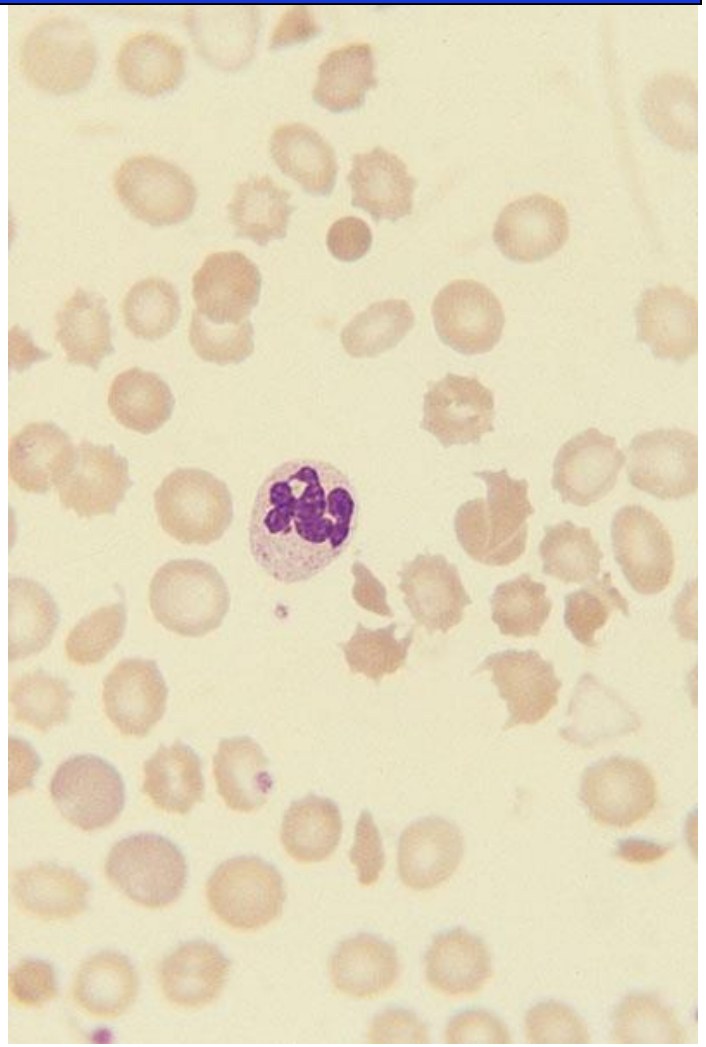
Bain BJ and Stubbs MJ (2015) Dengue fever in returning travellers. *Am J Hematol*, **90**, 263.

Thrombocytopenia + schistocytes – What could it be?

- TTP
- HUS
- HELLP
- Other microangiopathy
- Carcinomatosis

Haemolytic uraemic syndrome

- Usually a child with preceding diarrhoea
- A blood film must **always** be done in any patient presenting with acute kidney injury



Bain BJ, 2014, Interactive Imagebank, 2nd Edn, Wiley-Blackwell

Thrombotic thrombocytopenic purpura

- This is an emergency

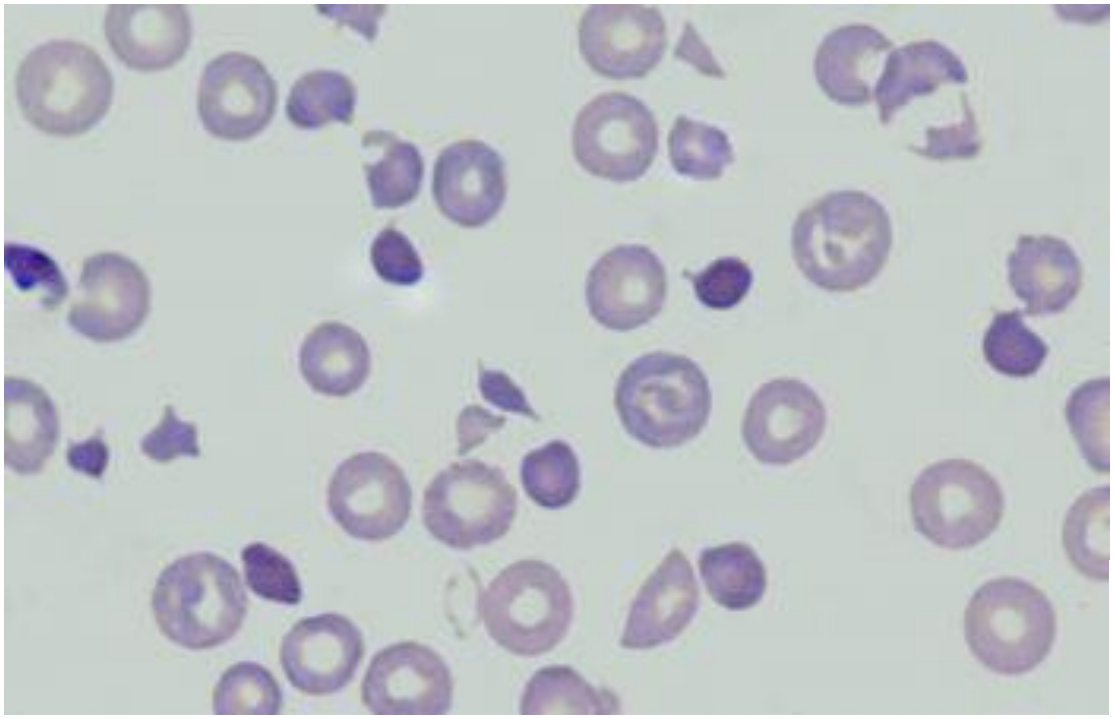
Thrombotic thrombocytopenic purpura

- Why is TTP an emergency?
- Because the mortality untreated is up to 90%
- Of 176 patients recorded in the SE England registry (2002–2006) 8.5% died
- Most patients who die, die before treatment is started
- Half of deaths are in the first 24 hours

Scully *et al*. (2008) *Br J Haematol*, **142**, 819–826.
Dutt & Scully (2015) *Br J Haematol*, **170**, 737–742.

Thrombotic thrombocytopenic purpura

- The blood film is very important



From Vallespi T and Garcia-Alonso L, Atlas of Blood cells and Blood Disease.

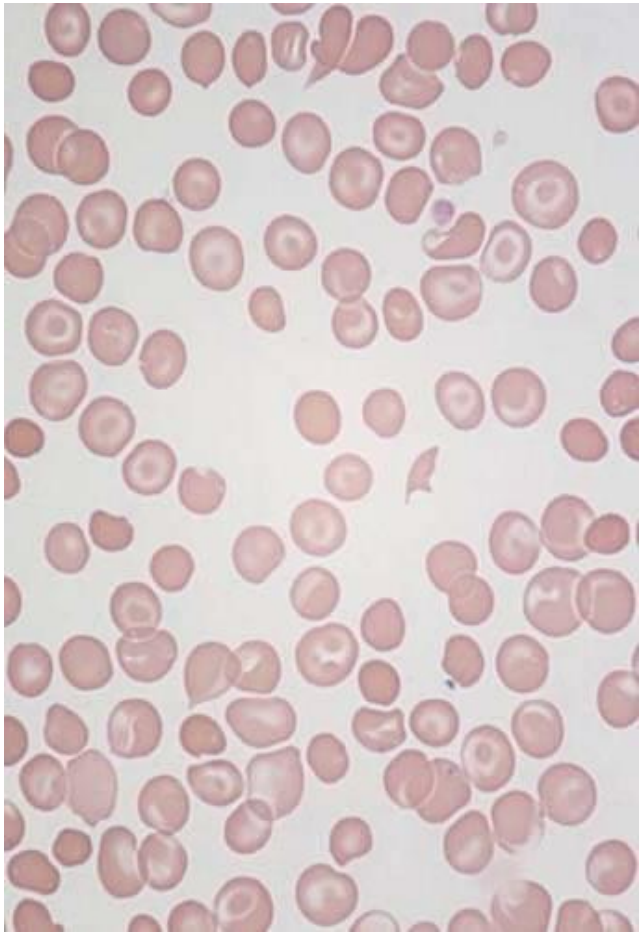
http://www.atlasbloodcells.es/

Thrombocytopenia + MAHA – not always easy

- A 26-year-old Indian woman with haemoglobin E disease
- Presented with epistaxis and petechiae
- Hb 88 g/l (usually ~96) and platelet count $<10 \times 10^9$/l
- Transfused 3 units of platelets
- Next morning became confused and lost consciousness

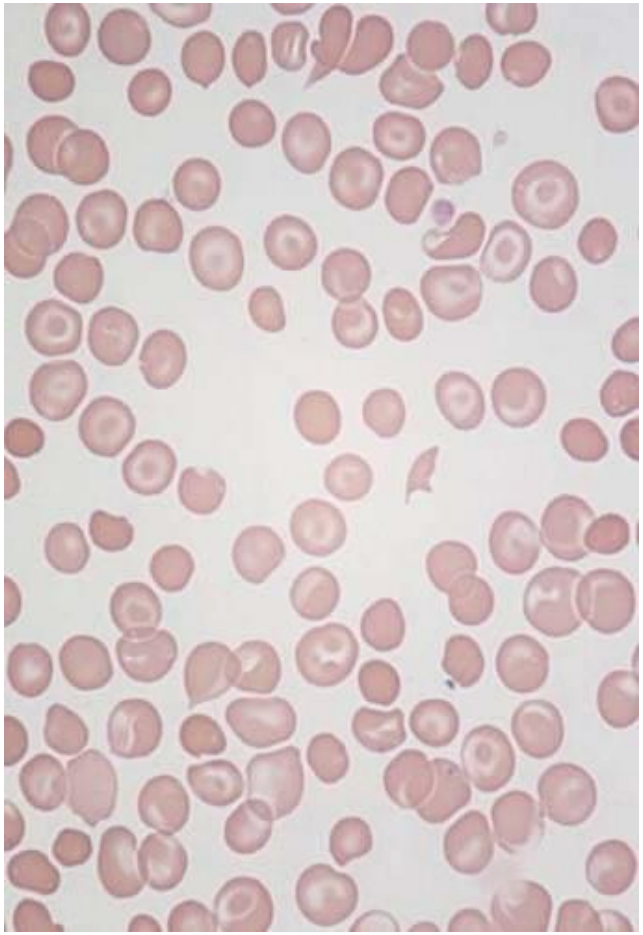
Thrombocytopenia + MAHA – not always easy

- What is the diagnosis?



Thrombocytopenia + MAHA – not always easy

- What is the diagnosis?
- Thrombotic thrombocytopenic purpura
- ADAMTS13 was < 5%



Hazarika B and Bain BJ (2012) Thrombotic thrombocytopenic purpura in a patient with hemoglobin E disease-the importance of timely examination of a blood film. *Am J Hematol*, **87**, 996.

Thrombotic thrombocytopenia purpura

- In a French data base of 423 patients there was delay in diagnosis in 20%
- This correlated with low numbers or even absent schistocytes (57% cf. 31% in promptly diagnosed cases)
- Overall 38% of patients had infrequent or undetectable schistocytes

Grall M, Azoulay E, Galicier L, Provôt F, Wynckel A, Poullin P *et al*. (2017) Thrombotic thrombocytopenic purpura misdiagnosed as autoimmune cytopenia: Causes of diagnostic errors and consequence on outcome. Experience of the French thrombotic microangiopathies reference centre. *Am J Hematol*, **92**, 381–387.

Thrombotic thrombocytopenic purpura

- Why does is matter?
  - Urgent plasma exchange is needed
  - Platelet transfusion should be avoided
- In a survey of 339 French patients with TTP, 43% received at least one platelet transfusion
- 81% of transfusion were given before the diagnosis was made, usually just because the count was low

Benhamou Y, Baudel JL, Wynckel A, Galicier L, Azoulay E, Provôt F et al. (2015) Are platelet transfusions harmful in acquired thrombotic thrombocytopenic purpura at the acute phase? Experience of the French thrombotic microangiopathies reference center. Am J Hematol, 90, E127-E129.

Thrombotic thrombocytopenic purpura

- 36% of patients deteriorated within 24 hours of platelet transfusion (neurological, myocardial infarction, cardiac arrest, death)
- Repeated platelet transfusions were associated with higher risk of post-transfusion deterioration
- Cerebral involvement was predictive

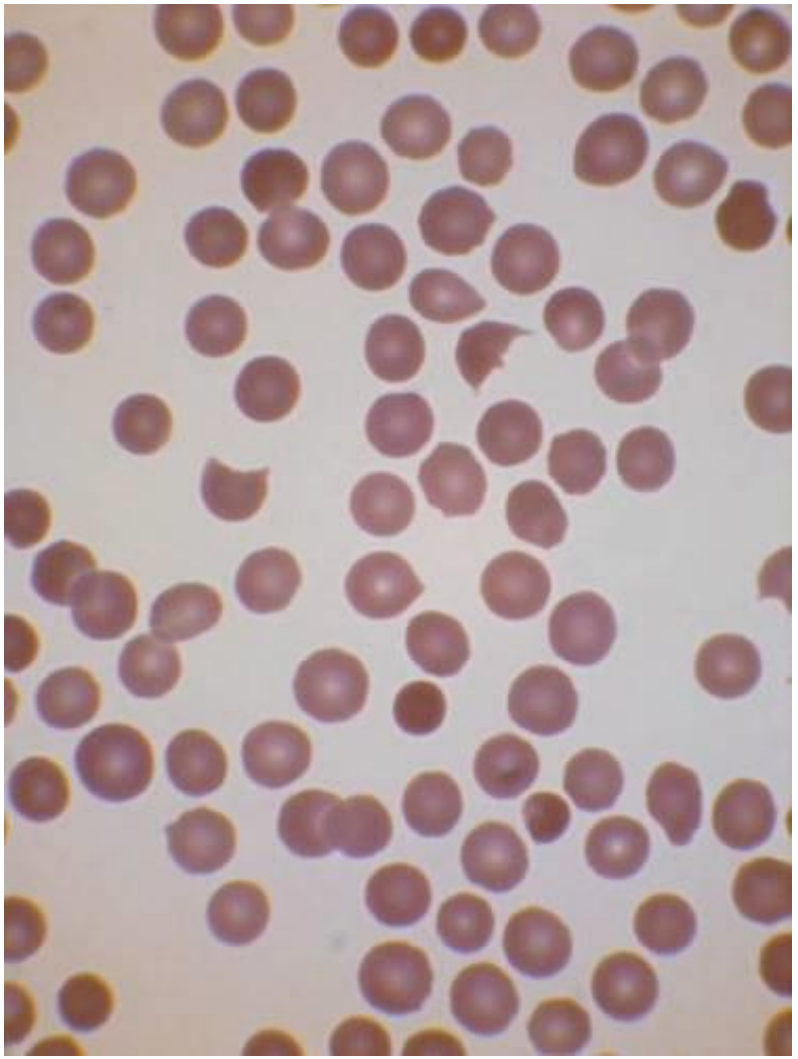
Benhamou Y, Baudel JL, Wynckel A, Galicier L, Azoulay E, Provôt F et al. (2015) Are platelet transfusions harmful in acquired thrombotic thrombocytopenic purpura at the acute phase? Experience of the French thrombotic microangiopathies reference center. Am J Hematol, 90, E127-E129.

Thrombocytopenia + MAHA

- A 31-year-old pregnant woman
- 26 weeks gestation
- Epigastric pain, diarrhoea and vomiting
- Hypertension and oedema
- Hb 89 g/l, platelet count 25 × 10^9/l

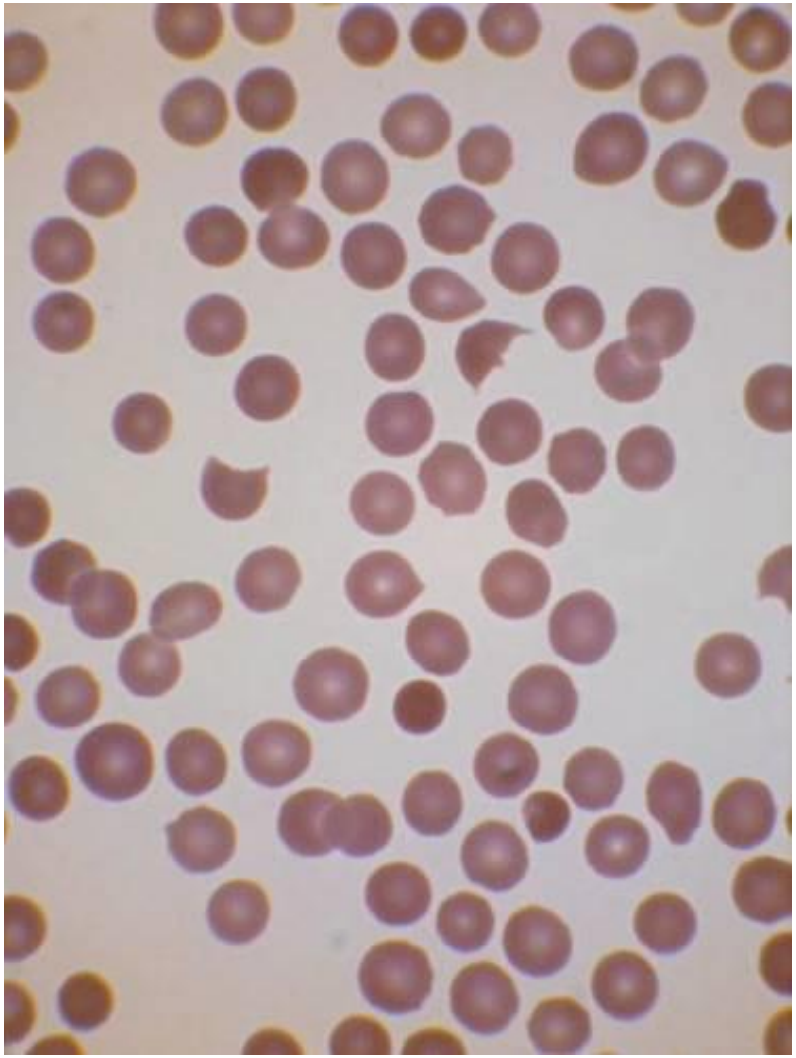
Thrombocytopenia + MAHA

- A 31-year-old pregnant woman
- What is the diagnosis?



Thrombocytopenia + MAHA

- A 31-year-old pregnant woman
- What is the diagnosis?
- HELLP syndrome – Haemolysis Elevated Liver enzymes Low Platelets



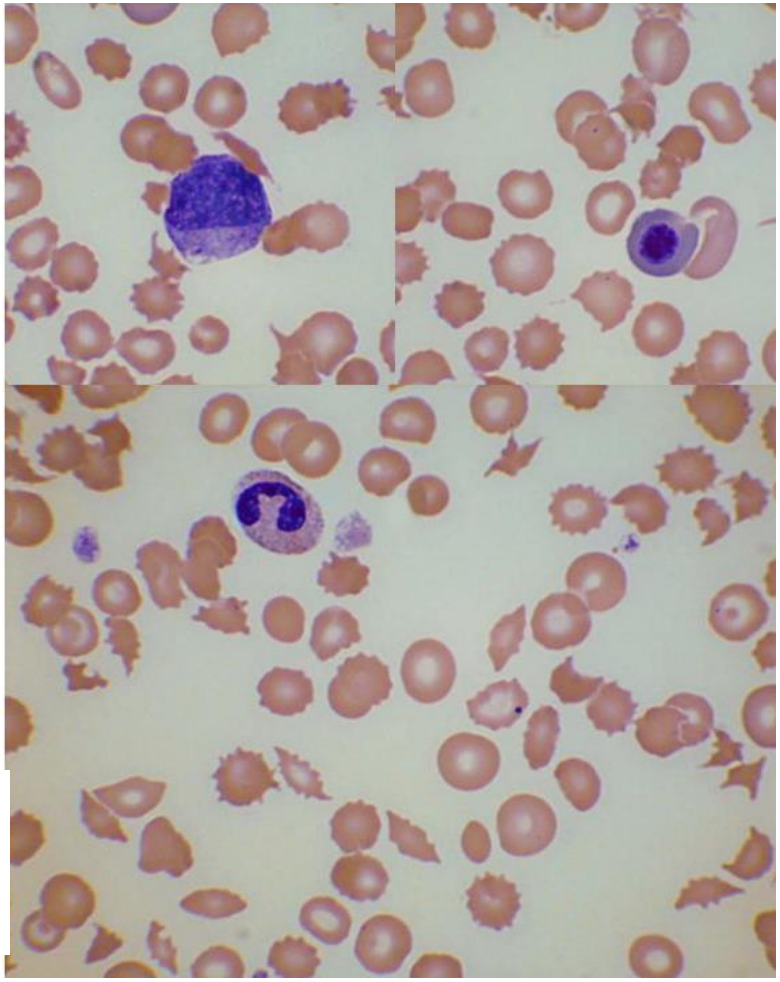
Bain BJ and Riches J (2010) Help with HELLP**.** *Am J Hematol*, **85**, 70.

Thrombocytopenia + MAHA

- A 71-year-old woman with a history of alcoholism and bilateral breast cancer
- Splenomegaly on CT scan
- WBC 26.6 × 10^9/l, Hb 90 g/l, platelet count 58 × 10^9/l

Thrombocytopenia + MAHA

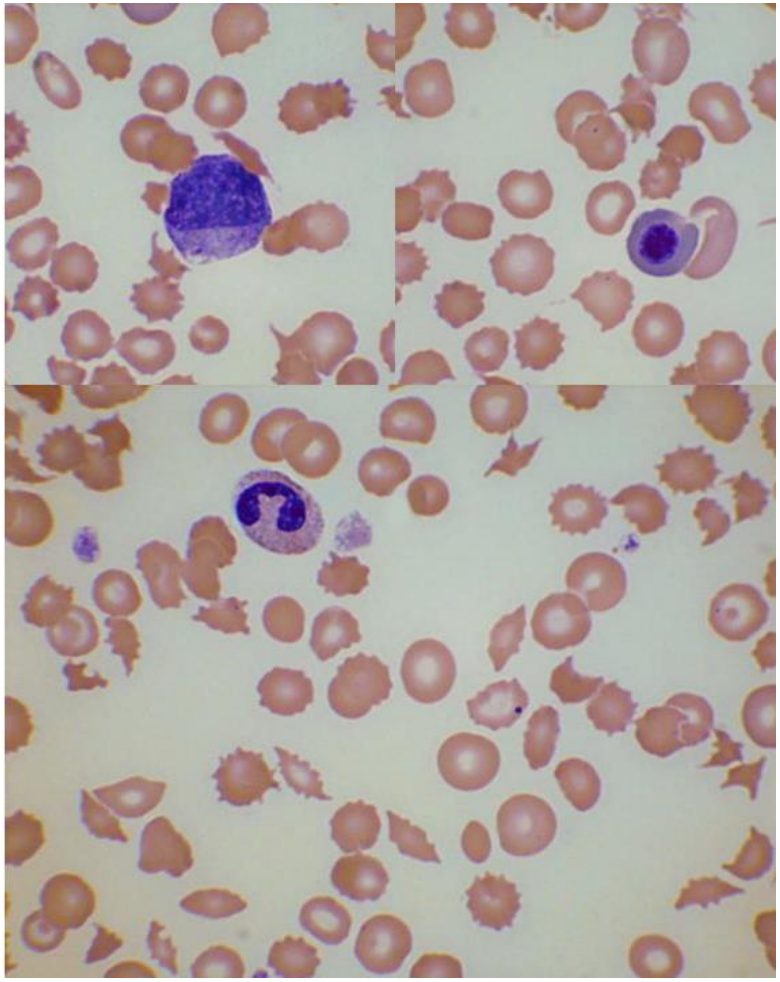
- A 71-year-old woman with previous breast cancer
- What is the diagnosis?



Bain BJ and Fosbury E (2009) Microangiopathic haemolytic anemia with hyposplenism. *Am J Hematol*, **84**, 242.

Thrombocytopenia + MAHA

- What is the diagnosis?
- Carcinocythaemia
- Bone marrow infiltration
- Hyposplenism
- Thrombotic microangiopathy



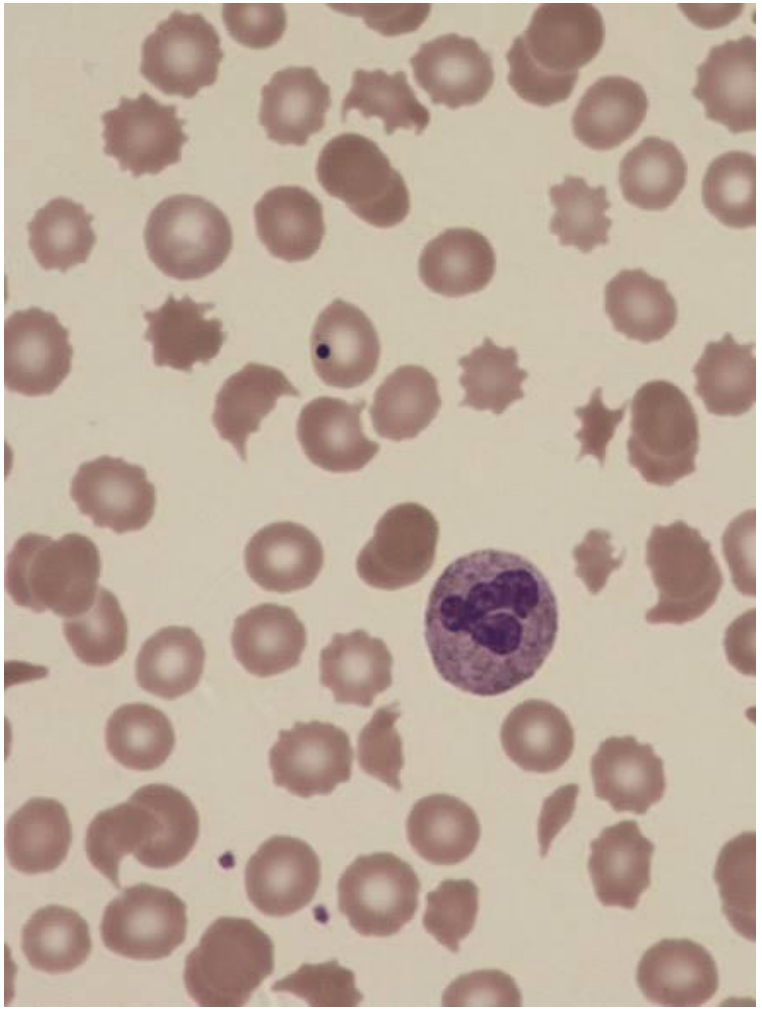
Bain BJ and Fosbury E (2009) Microangiopathic haemolytic anemia with hyposplenism. *Am J Hematol*, **84**, 242.

Thrombocytopenia + MAHA

- A 62-year-old woman with carcinoma of the pancreas being treated with gemcitabine
- Previous splenectomy and radiotherapy
- Acute kidney injury
- WBC 9.5 × 10^9/l, Hb 91 g/l, platelet count 89 × 10^9/l
- ADAMTS13 87%

Thrombocytopenia + MAHA

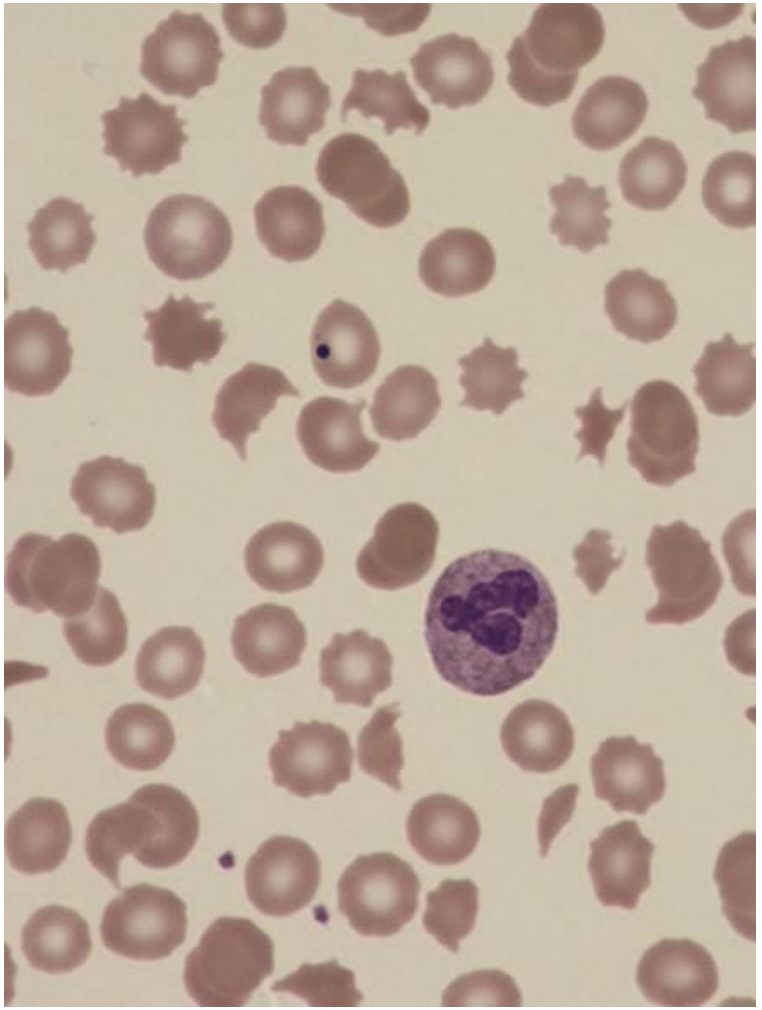
- A 68-year-old woman with carcinoma of the pancreas
- How many factors can we see interacting here?



Erblich T, Hill PD, Tomlinson J and Bain BJ (2015) The complex morphology of acute kidney injury with microangiopathic hemolytic anemia and hyposplenism. *Am J Hematol*, **90**, 674.

Thrombocytopenia + MAHA

- How many factors can we see interacting here?
- Hyposplenism – previous splenectomy
- Gemcitabine-induced microangiopathy
- Acute kidney injury



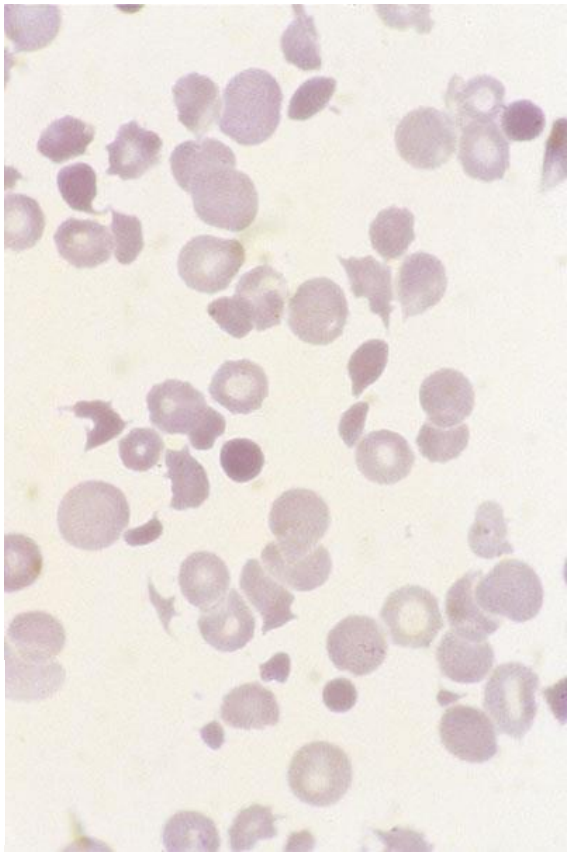
Erblich T, Hill PD, Tomlinson J and Bain BJ (2015) The complex morphology of acute kidney injury with microangiopathic hemolytic anemia and hyposplenism. *Am J Hematol*, **90**, 674.

Thrombocytosis and falsely normal or overestimated platelet counts

- Is the count true?
  - Red cell fragments
  - White cell fragments
  - Fungi
  - Cryoglobulin
- Are there clues as to aetiology?
  - Basophilia
  - Polycythaemia
  - Leucoerythroblastic

Pseudothrombocytosis or falsely normal or overestimated platelet count

- Red cell fragments due to snakebite

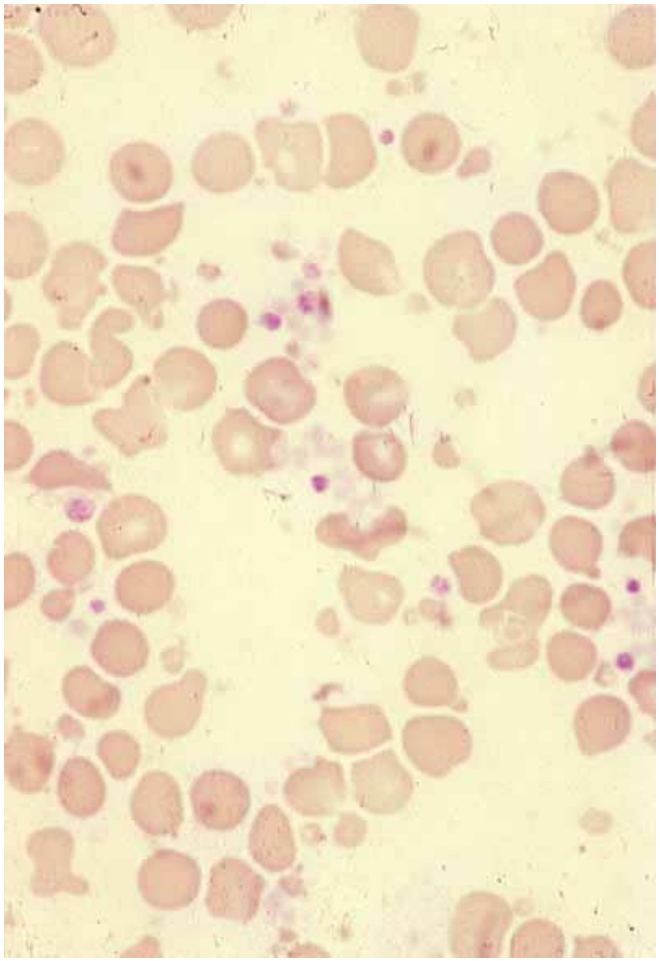


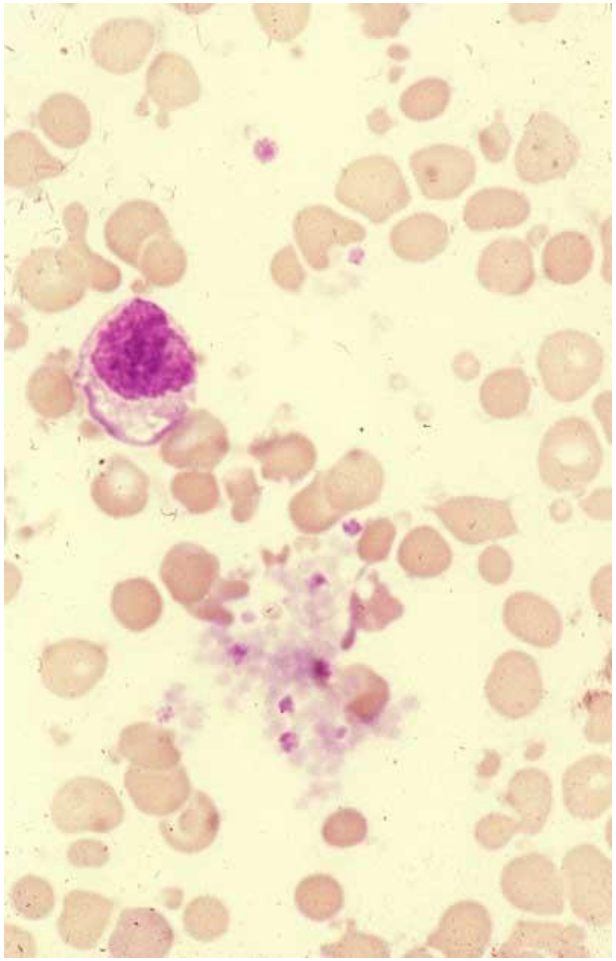
Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley-Blackwell, Oxford

Pseudothrombocytosis

- 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 × 10^9/l
  - Hb 107 g/l
  - MCV 82.6 fl
  - RDW 20.6
  - Platelet count 1539 × 10^9/l

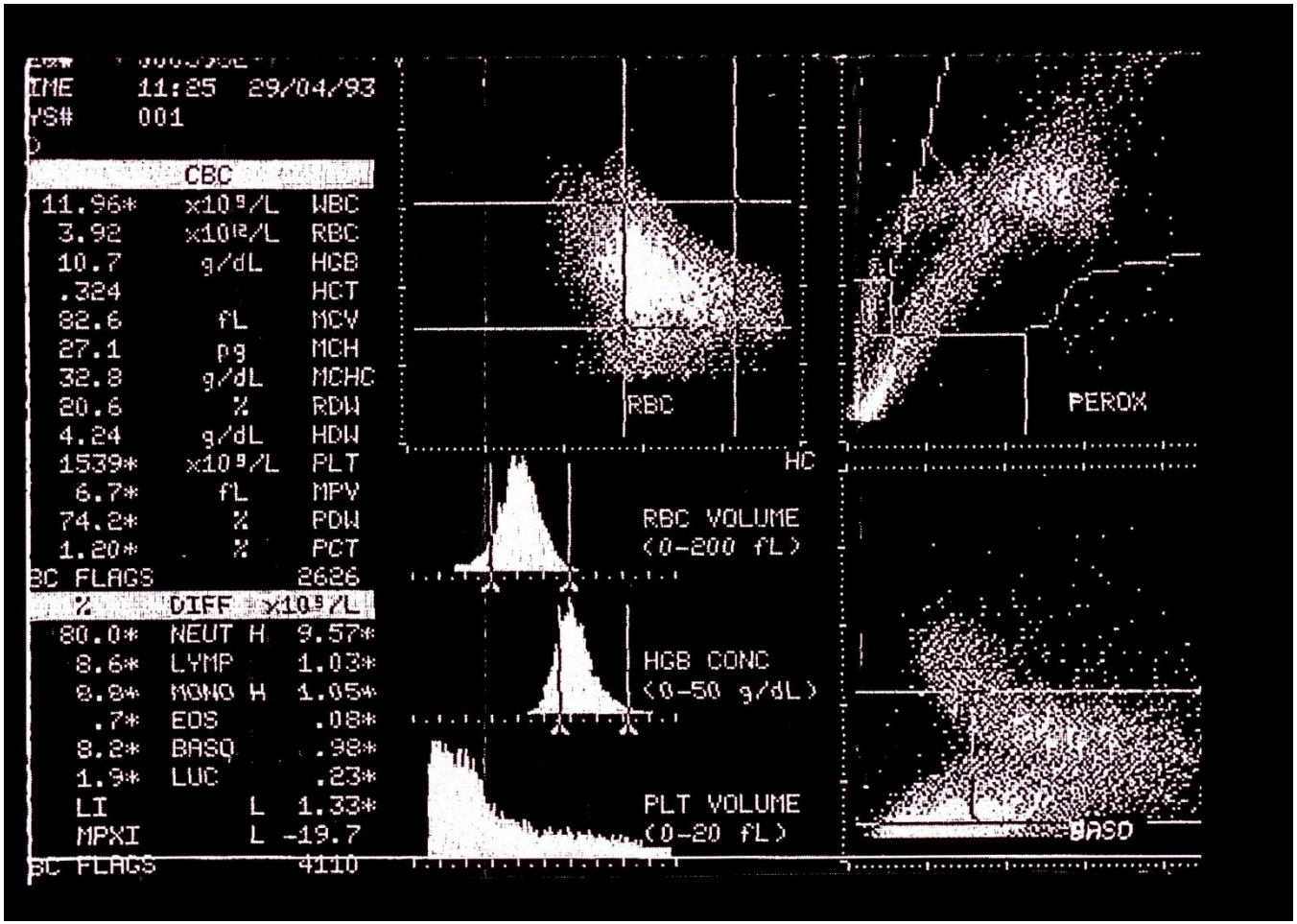
Pseudothrombocytosis





Blood film

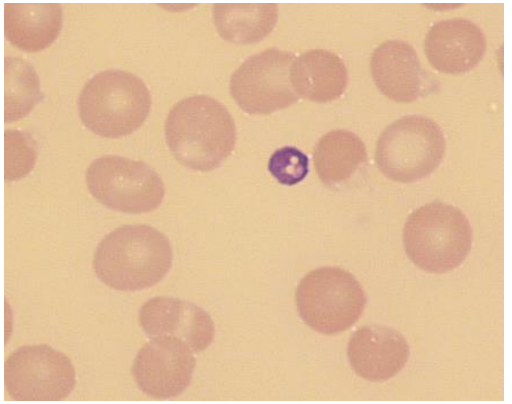
Pseudothrombocytosis

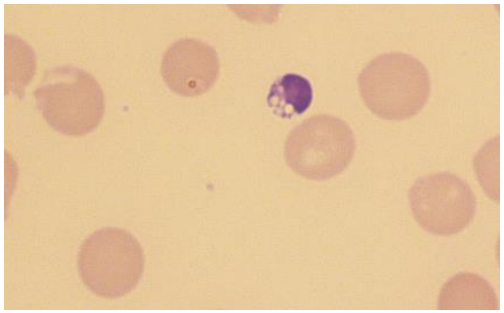


H2 scatterplots

Pseudothrombocytosis or falsely normal or overestimated platelet counts

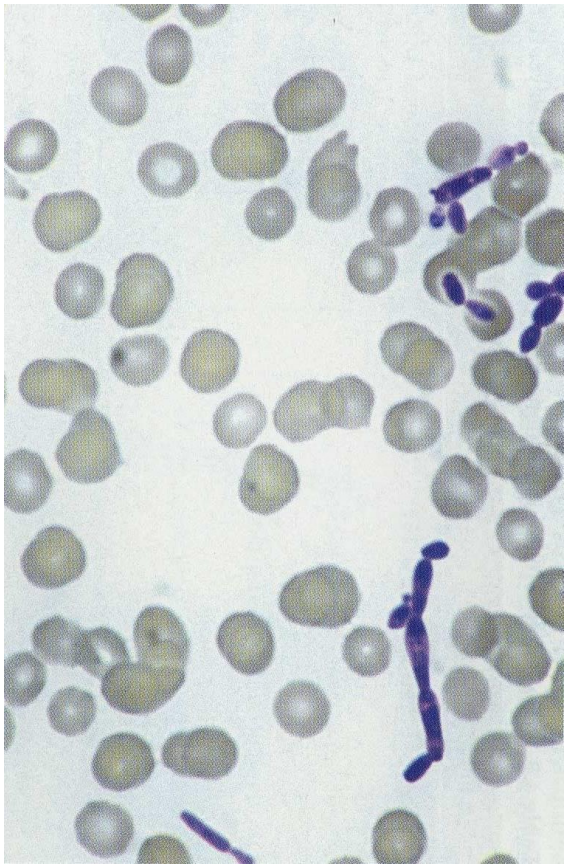
- White cell fragments
  - Leukaemia
  - Lymphoma
- Pseudoplatelets in Burkitt lymphoma





Pseudothrombocytosis or falsely normal count

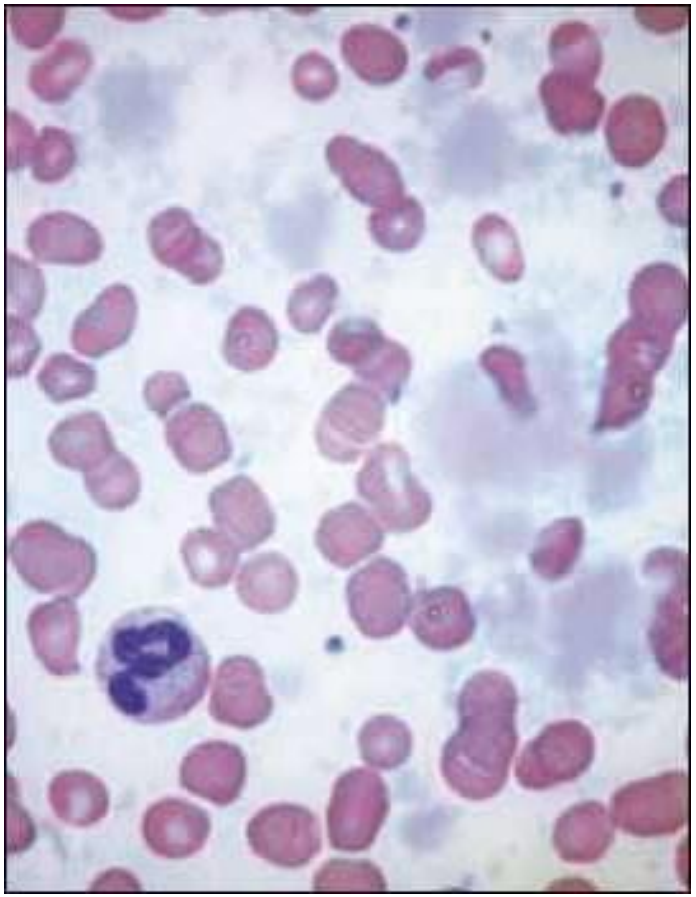
- Fungi – *Candida glabrata*



Bain BJ (2015) *Blood Cells* 5th Edn Wiley-Blackwell, Oxford.

Pseudothrombocytosis

Cryoglobulinaemia



Bain BJ (2005) Diagnosis from the blood smear. *N Engl J Med*, **353**, 498-507.

True thrombocytosis

- Inherited
  - Mutation in the thrombopoietin gene (*THPO*)
  - Mutation in the *MPL* gene, encoding the receptor for thrombopoietin
  - *MPL*-Baltimore polymorphism in the MPL gene (7% of African Americans)
  - *JAK2* mutation (e.g. *JAK2* V617I and *JAK2* R564Q)
  - Other
- Except for *MPL*-Baltimore, all very rare

True thrombocytosis

Acquired

- Reactive
  - Infection
  - Inflammation
  - Malignancy
  - Iron deficiency
  - POEMS syndrome
- Redistributive
  - Hyposplenism
- Neoplastic

True thrombocytosis – neoplastic causes

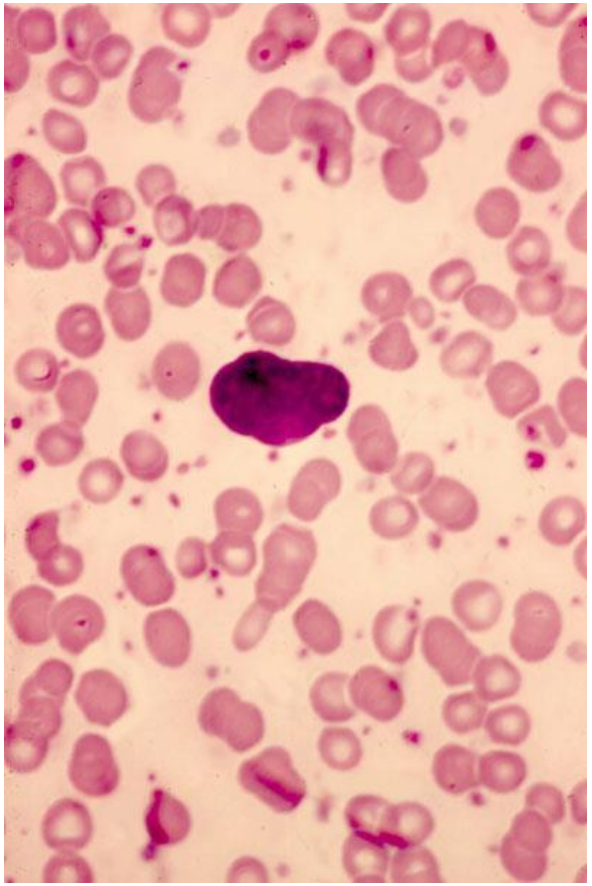
- Myeloproliferative neoplasms
  - Chronic myeloid leukaemia
  - Polycythaemia vera
  - Essential thrombocythaemia
  - Prefibrotic stage of primary myelofibrosis
- Myelodysplastic/myeloproliferative neoplasms
  - Refractory anaemia with ring sideroblasts and thrombocytosis

True thrombocytosis – neoplastic causes

- Acute myeloid leukaemia (rarely)
  - Associated with t(3;3) or inv(3)
- Transient abnormal myelopoiesis
- Myelodysplastic syndrome
  - 5q- syndrome

True thrombocytosis

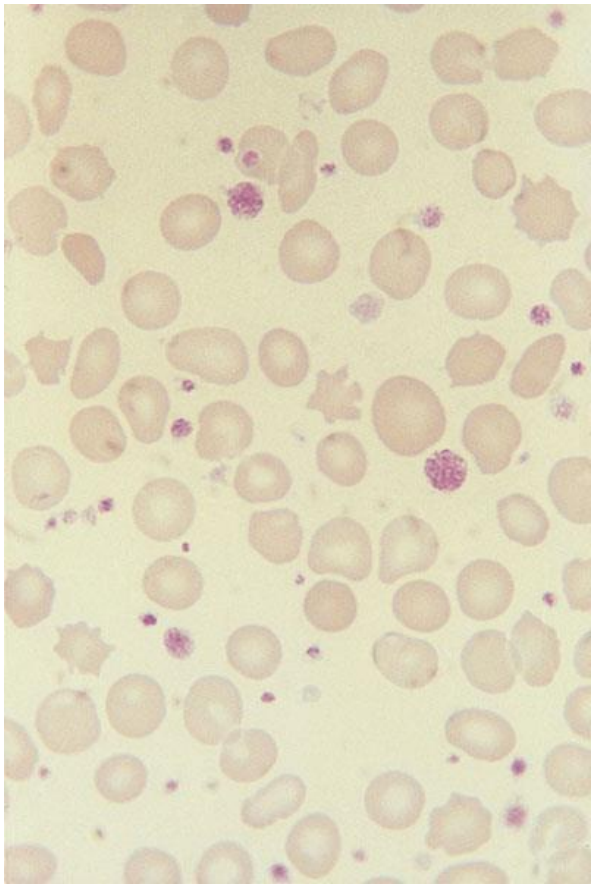
- Megakaryoblastic transformation of CML



Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley-Blackwell, Oxford

True thrombocytosis

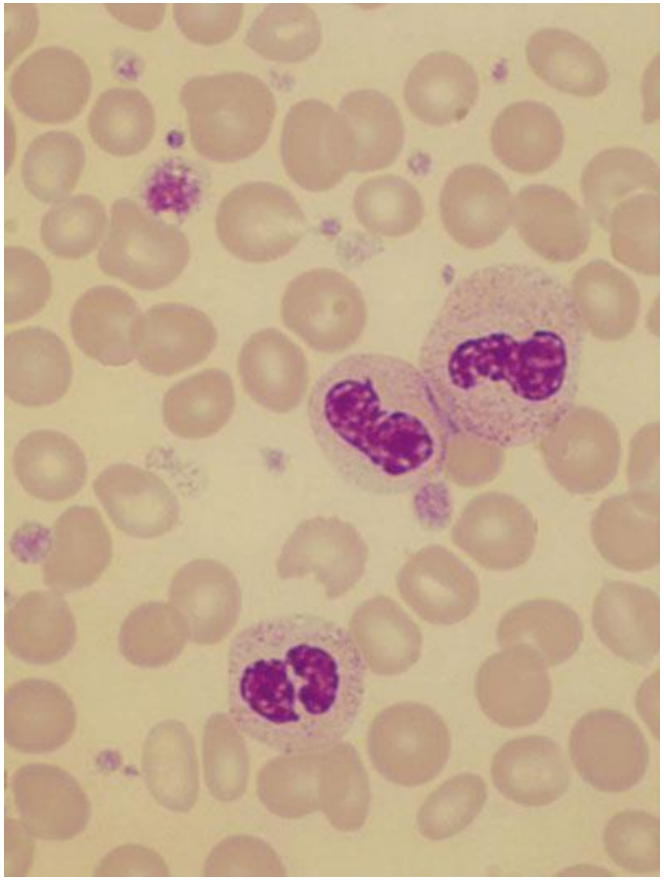
- Essential thrombocythaemia



Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley-Blackwell, Oxford

True thrombocytosis

- Refractory anaemia with ring sideroblasts and thrombocytosis



Bain BJ (2014) Interactive Haematology Imagebank, 2nd Edn, Wiley-Blackwell, Oxford

Morphology to the rescue

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The End

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