The Neglected Red Cell

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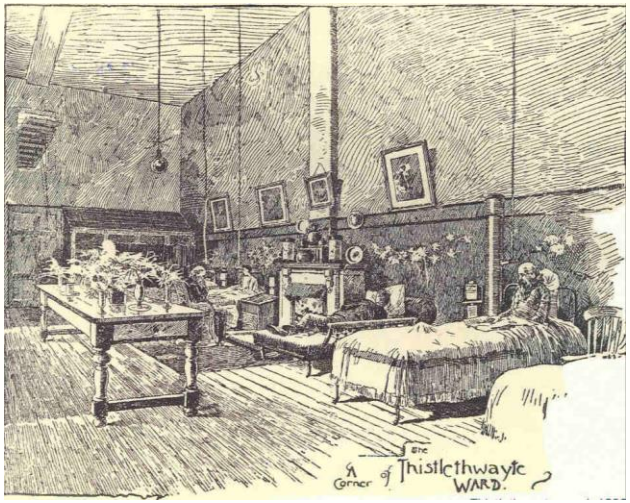
NEQAS, Birmingham, 2018



PDF

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Why is the red cell neglected?

- Remarkable advances have been in the diagnosis and management of leukaemias and lymphomas in recent decades
- This has tended to dominate haematology so that other aspects are neglected

Why is the red cell neglected?

- The intensive nature of leukaemia management means that haematologists are much less often in the laboratory with the risk that they will become deskilled
- However therapeutic advances (e.g. in sickle cell disease and PNH) and major advances in diagnosis or red cell disorders mean that more attention is now being paid to the red cell

Why is red cell diagnosis important?

- Specific treatment may be indicated (or contraindicated)
  - Plasma exchange for TTP
  - Valve replacement for mechanical haemolytic anaemia
  - Eculizumab for PNH
  - Corticosteroids for AIHA
  - Splenectomy for severe haemolytic anaemia (but contraindicated in hereditary stomatocytosis)

How should we diagnose red cell disorders?

- Medical history
- Family history
- Physical examination
- Blood count (± reticulocyte count)
- Blood film
- Further tests as indicated

How should we diagnose red cell disorders?

- Medical history
- Family history
- Physical examination
- Blood count
- Blood film
- Further tests as indicated

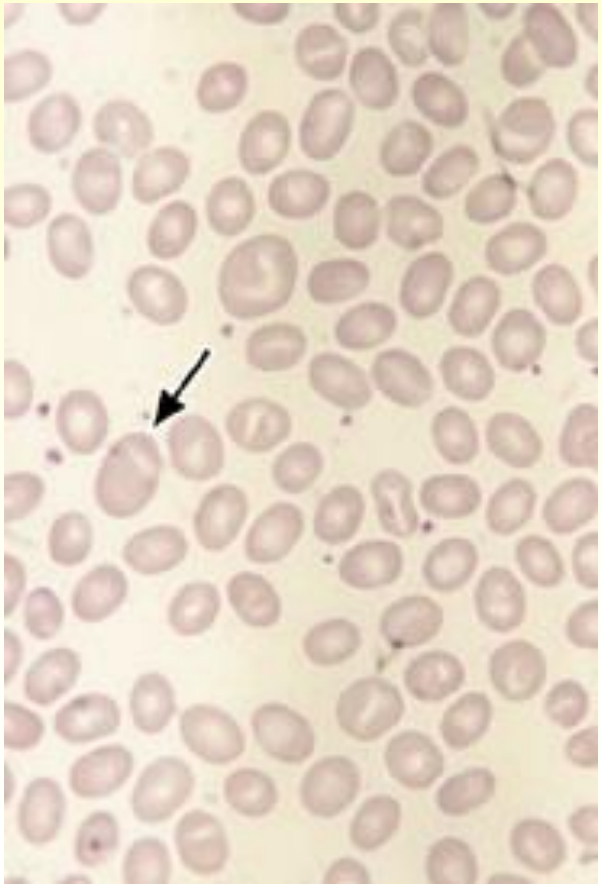
Diagnosis

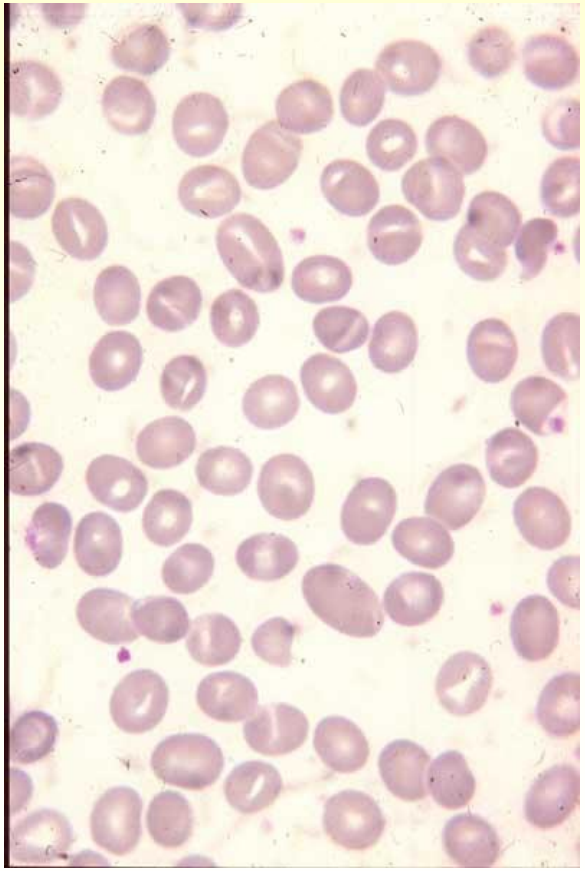
Sometimes the blood film suggests a specific diagnosis

Lund Historical Museum/
University Museum

Indicating a specific diagnosis (i)

South-east Asian ovalocytosis





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Indicating a specific diagnosis

South-east Asian ovalocytosis

- Mutation in *SLC4A1*
- Protects against malaria
- Does this diagnosis matter?

Indicating a specific diagnosis

South-east Asian ovalocytosis

- Mutation in *SLC4A1*
- Protects against malaria
- Does this diagnosis matter?
- Homozygosity causes fetal hydrops and intrauterine death

Picard V, Proust A, Eveillard M, Flatt JF, Couec ML, Caillaux G et al. (2014) Homozygous Southeast Asian ovalocytosis is a severe dyserythropoietic anemia associated with distal renal tubular acidosis. *Blood*, **123**, 1963-1965.

Indicating a specific diagnosis

South-east Asian ovalocytosis

- Mutation in *SLC4A1*
- Protects against malaria
- Does this diagnosis matter?
- Heterozygosity is not of any significance in the adult
- Causes neonatal haemolytic anaemia and hyperbilirubinaemia in about half of infants

Laosombat V, Dissaneevate S, Wongchanchailert M and Satayasevanaa B (2005) Neonatal anemia associated with Southeast Asian ovalocytosis. *Int J Hematol*, 82, 201–205

Indicating a specific diagnosis (ii)

A diagnostic problem

- 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)

Indicating a specific diagnosis

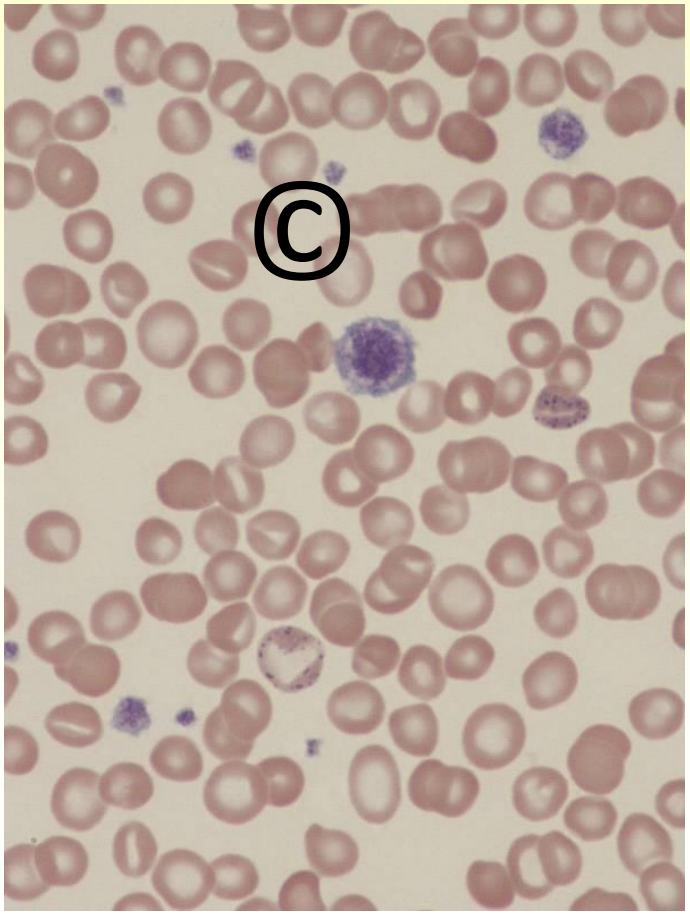
A diagnostic problem

- 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)

Indicating a specific diagnosis

A diagnostic problem

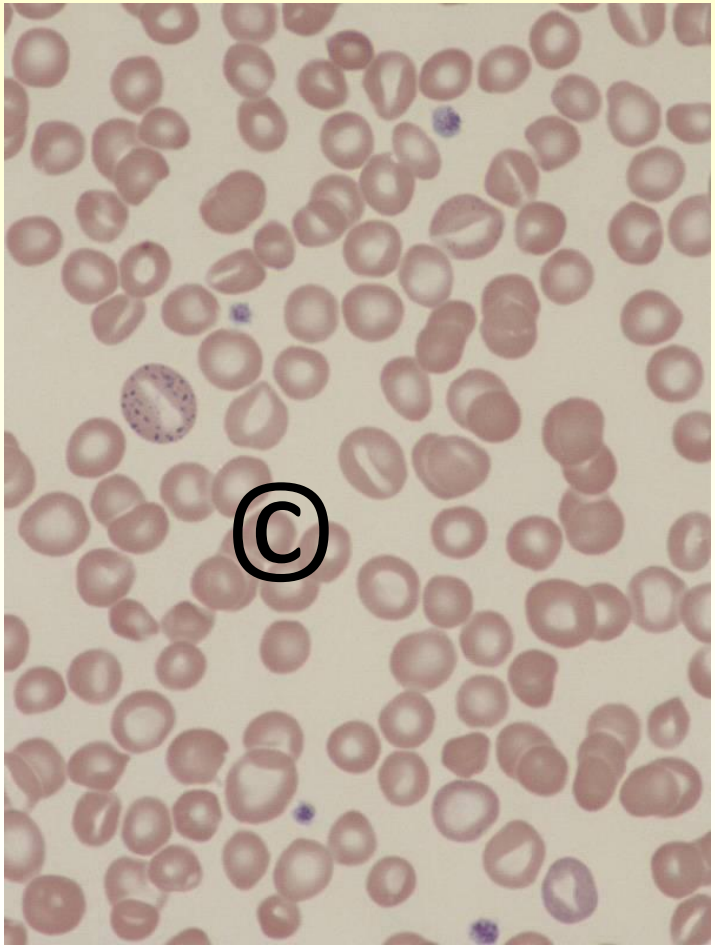
- Blood film, in addition to the features of β thalassaemia trait, showed giant platelets and stomatocytosis
- What diagnosis do you suggest?



Indicating a specific diagnosis

A diagnostic problem

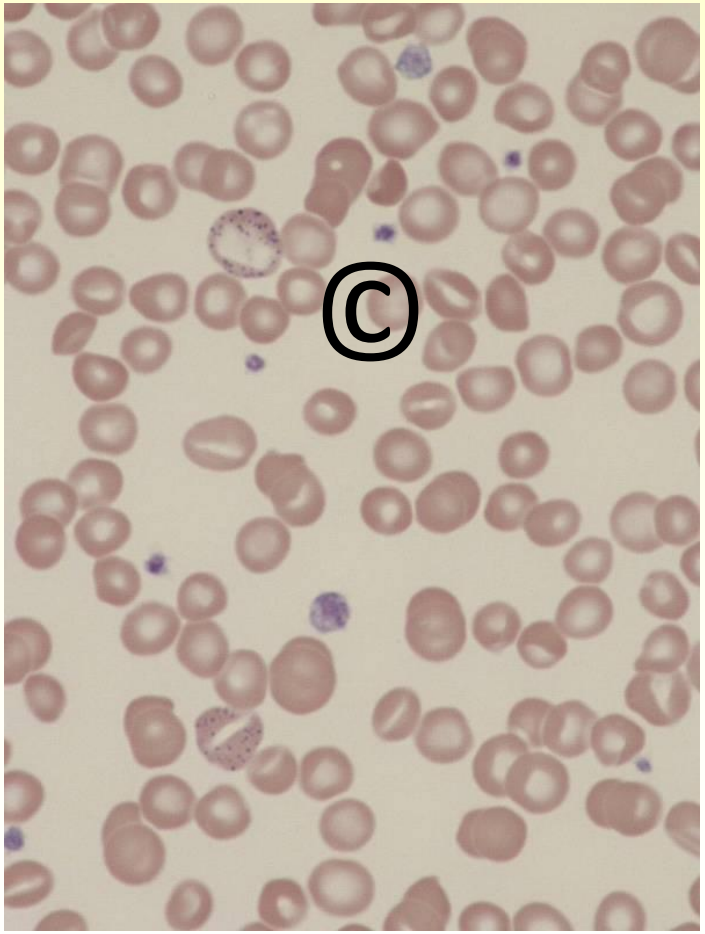
- What diagnosis do you suggest?
- Phytosterolaemia (also known as sitosterolaemia)
- An AR condition (mutated *ABCG5* or *ABCG8* gene) with increased absorption of plant and other sterols



Indicating a specific diagnosis

A diagnostic problem

- Does this diagnosis matter?



Indicating a specific diagnosis

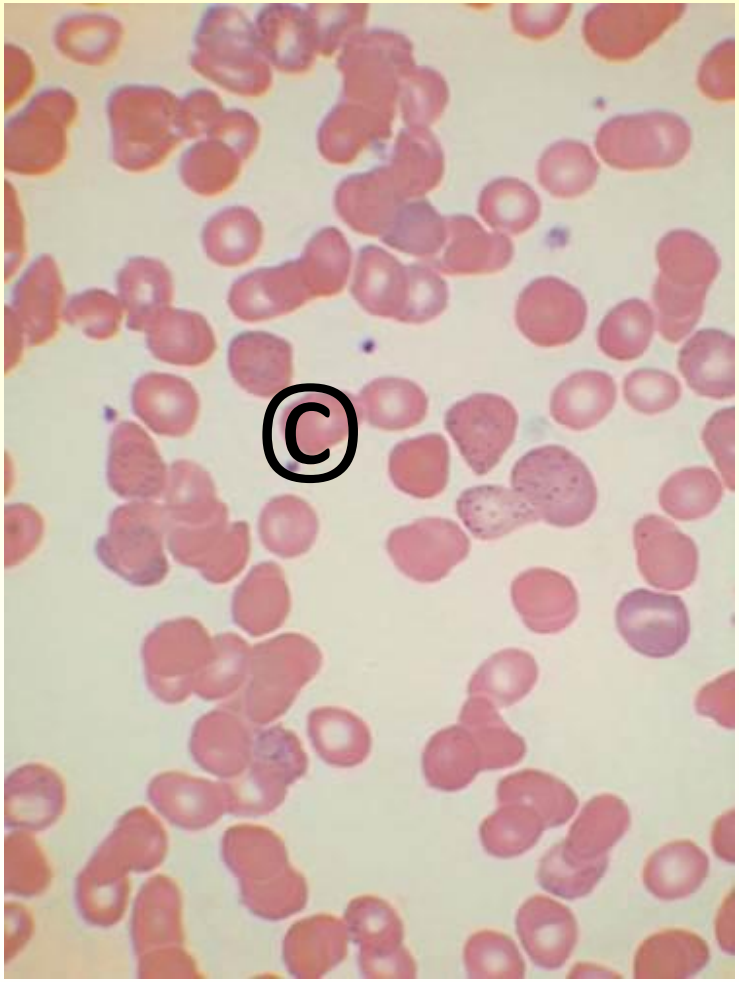
A diagnostic problem

- Does this diagnosis matter?
- Yes, 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 and treatment also in his brother

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

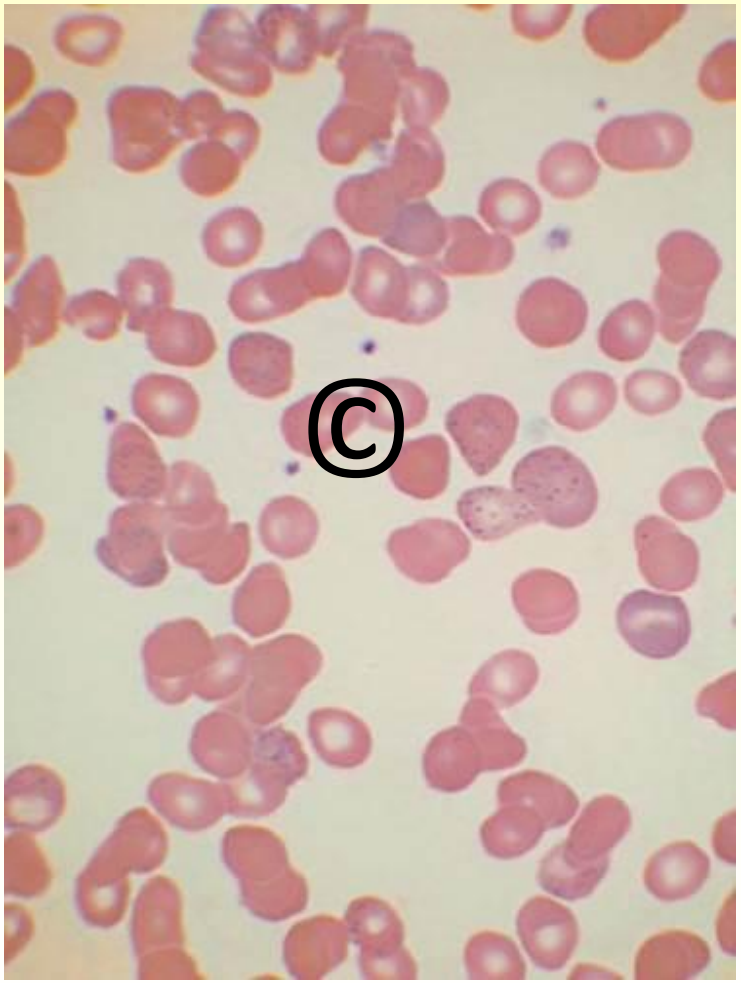
Indicating a specific diagnosis (iii)

- A 21-year-old woman from Kuwait
- Anaemic since birth and has sometimes needed transfusion
- Hb 81 g/l, reticulocytes 13.7%
- What diagnosis would you suggest?



Indicating a specific diagnosis

- What diagnosis do you suspect?
- Pyrimidine 5′ nucleotidase deficiency



Al-Jafar HA, Layton DM, Robertson L, Escuredo E and Bain BJ (2013) Diagnosis of pyrimidine 5'-nucleotidase deficiency suspected from a blood film. *Am J Hematol*, **88**,1089.

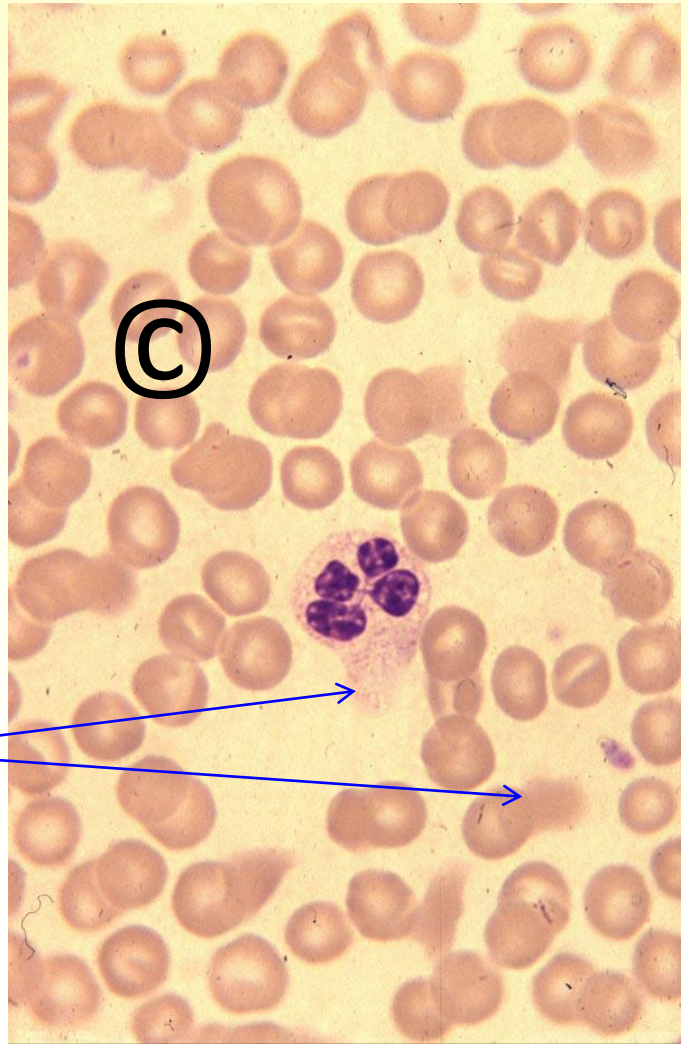
Sometimes the automated instrument output and the information from the blood film need to be integrated

A blood count that needs validation

- A 52-year-old woman with a heavy alcohol intake
- FBC (Coulter) WBC 5.8 × 10^9/l, RBC 4.37 × 10^{12}/l, Hb 175 g/l, Hct 0.42 l/l, MCV 95 fl, MCH 40 pg, MCHC 421 g/l, platelets 322 × 10^9/l
- Is she polycythaemic?

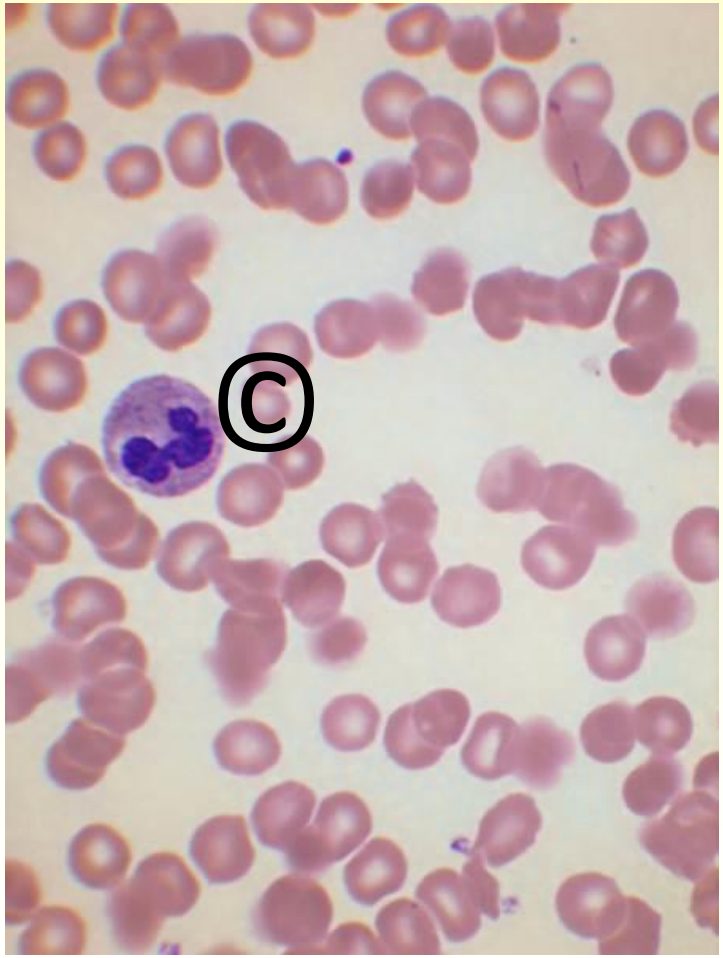
A blood count that needs validation

- WBC 5.8 × 10^9/l, RBC 4.37 × 10^{12}/l, Hb 175 g/l, Hct 0.42 l/l, MCV 95 fl, MCH 40 pg, MCHC 421 g/l
- What is the explanation?
- 'Fuzzy' red and white cells
- Hyperlipidaemia
- Factitious results



Another example

- Blood film made because of an MCHC of 367 g/l (316–349)
- Triglycerides 26.76 mmol/l (0–2)



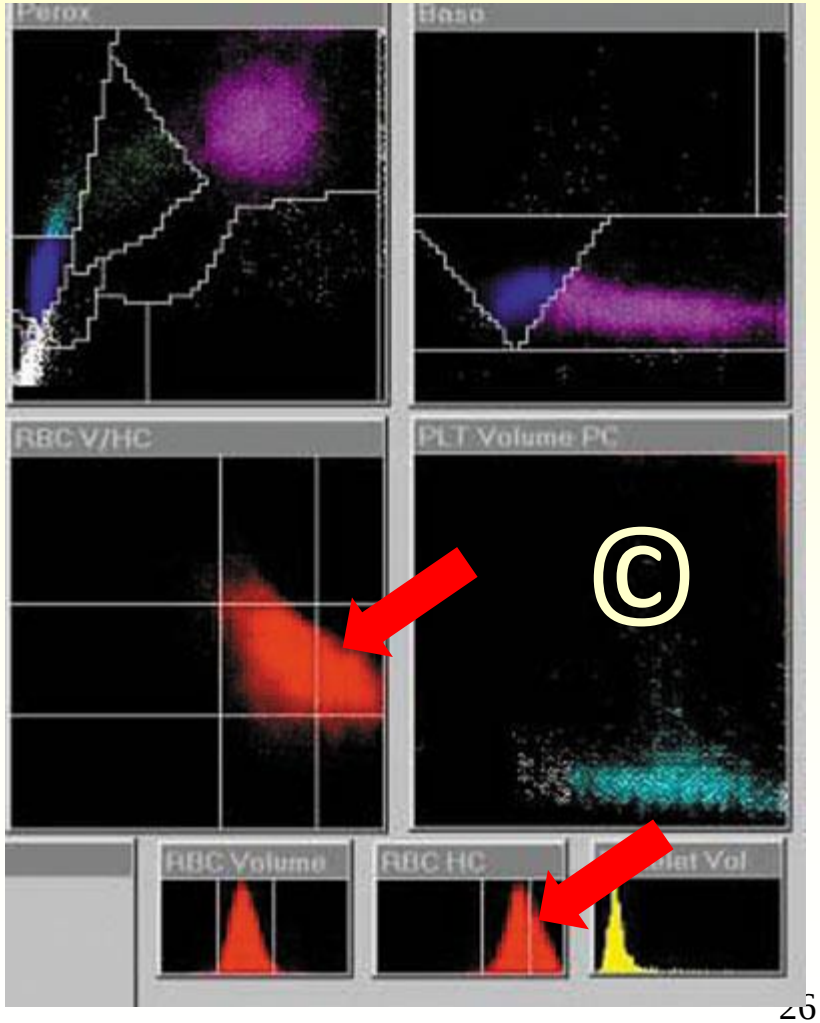
Miller CE, Hirani B and Bain BJ (2013) Hyperlipidemia revealed by erythrocyte morphology. *Am J Hematol*, **88**, 625.

MCHC ↑ or Hyperchromia flag

- Spherocytosis
- Irregularly contracted cells
- Sickle cells
- Occasionally hereditary elliptocytosis

MCHC ↑or Hyperchromia flag (i)

- What is the explanation?



MCHC ↑ or Hyperchromia flag

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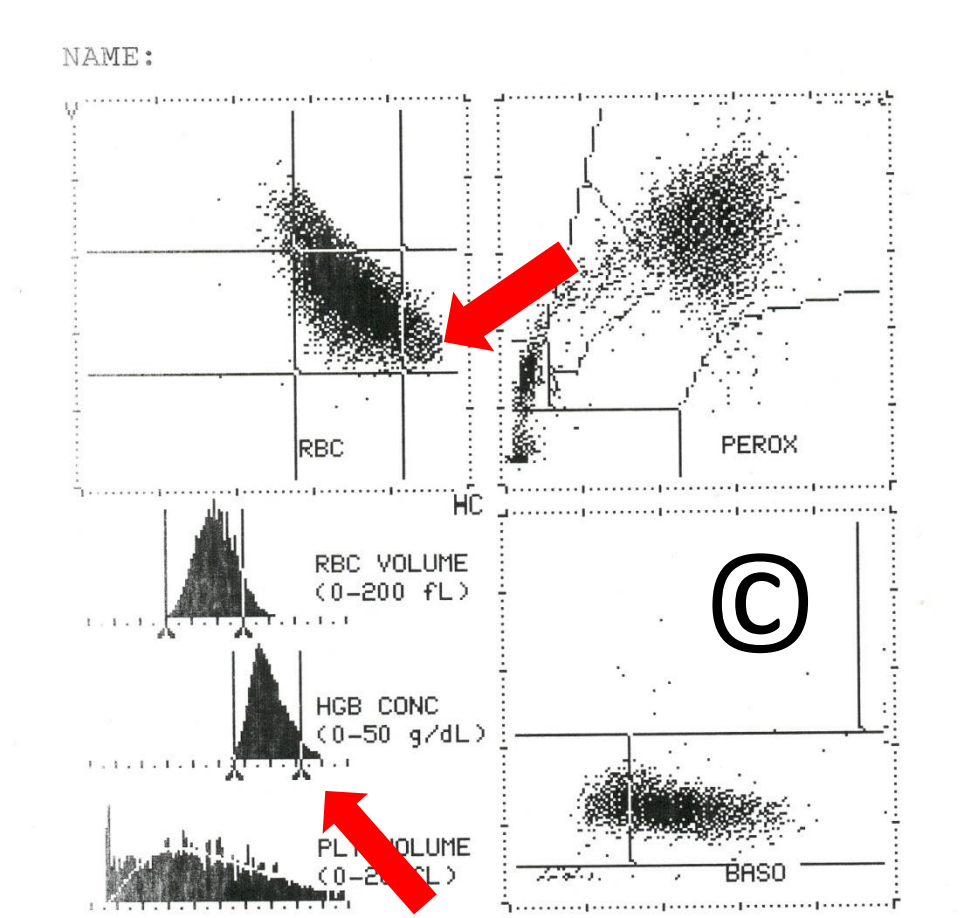
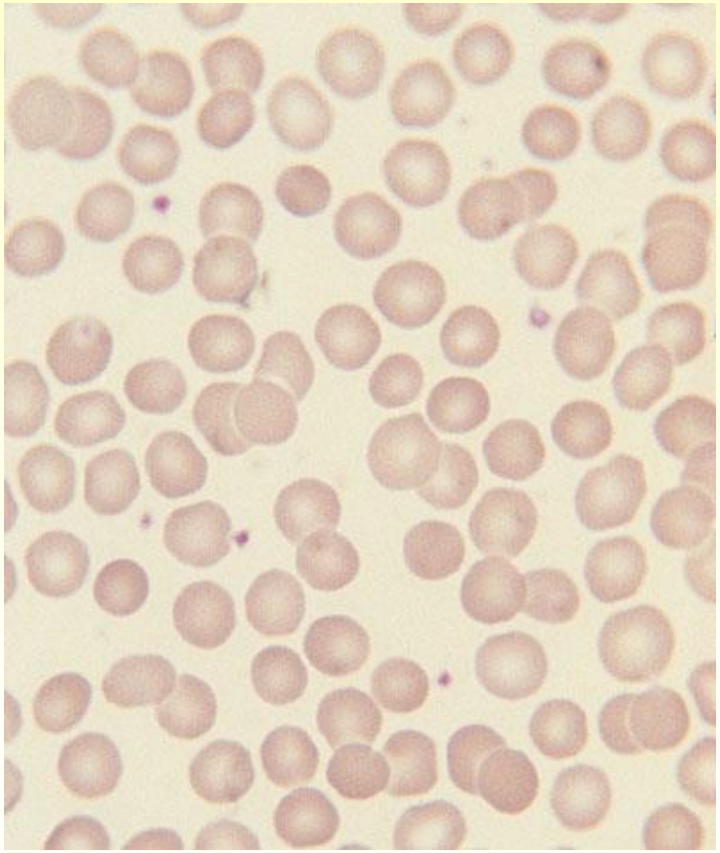
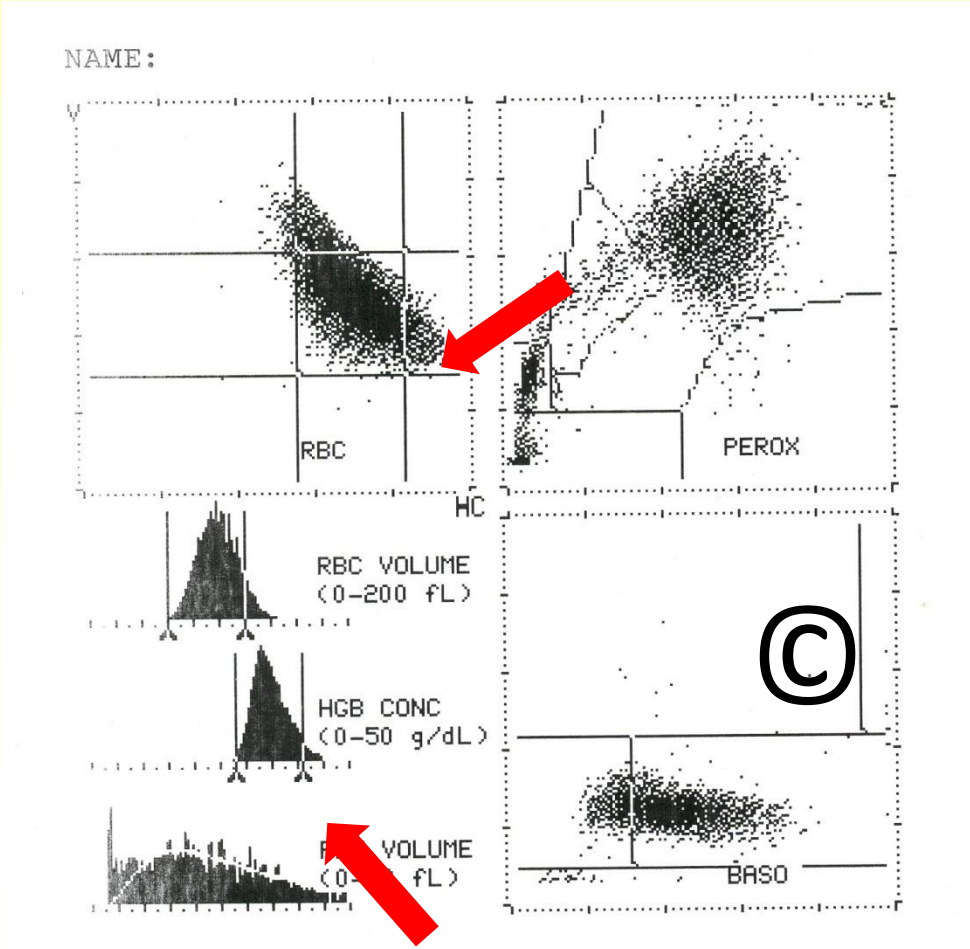


MCHC ↑ or Hyperchromia flag

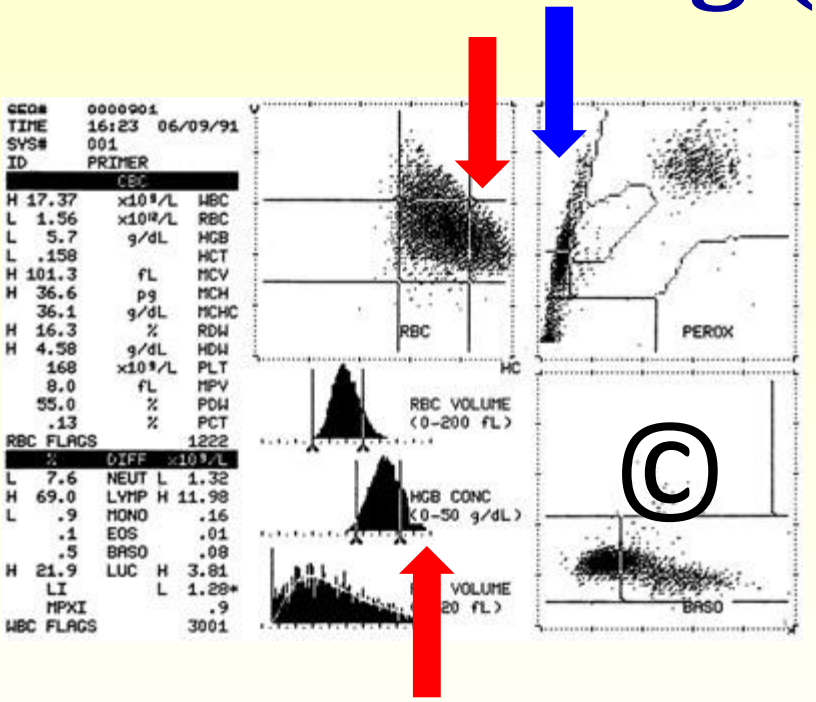




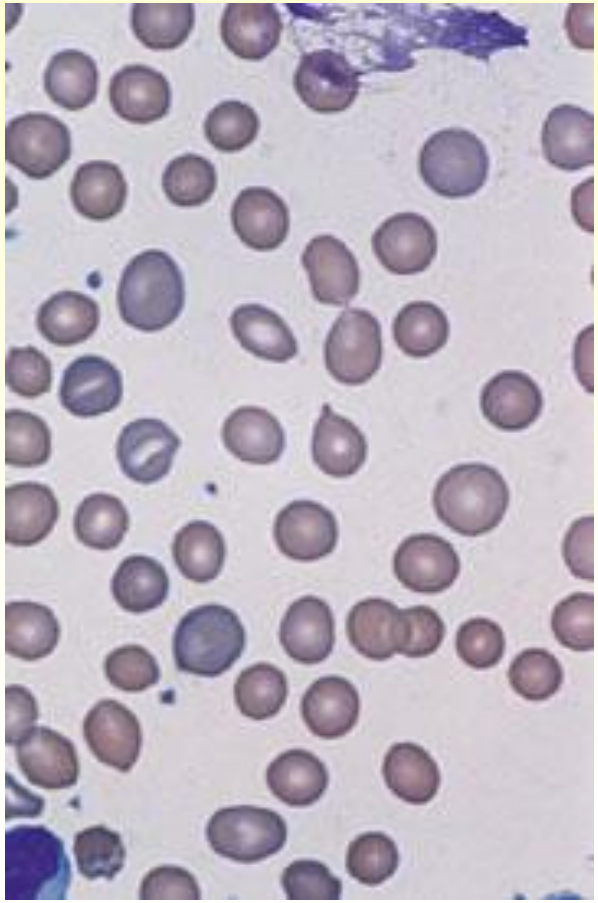
- Hereditary spherocytosis

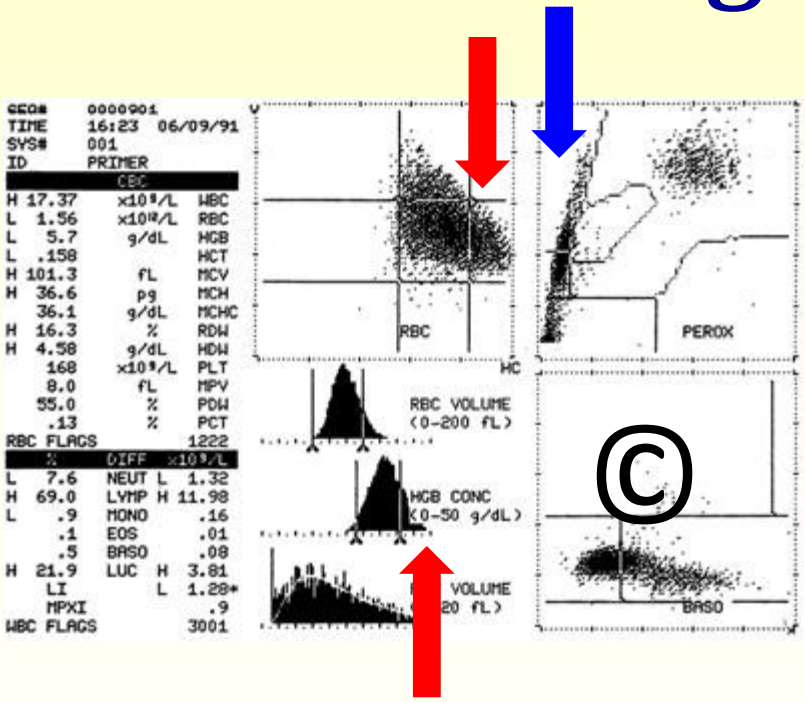
MCHC ↑or Hyperchromia flag (i)

- What is the explanation this time in an elderly man?



MCHC ↑ or Hyperchromia flag

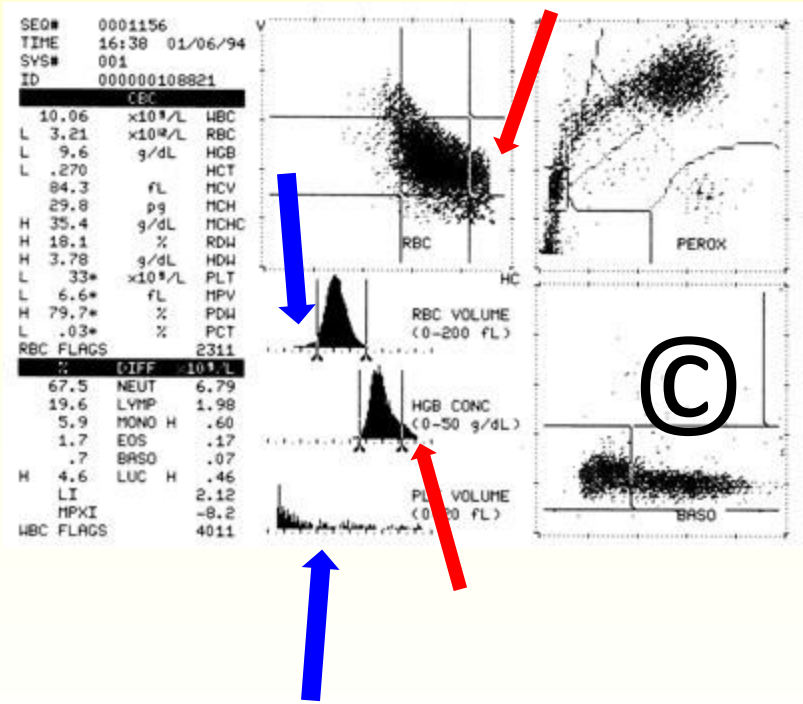




AIHA in CLL

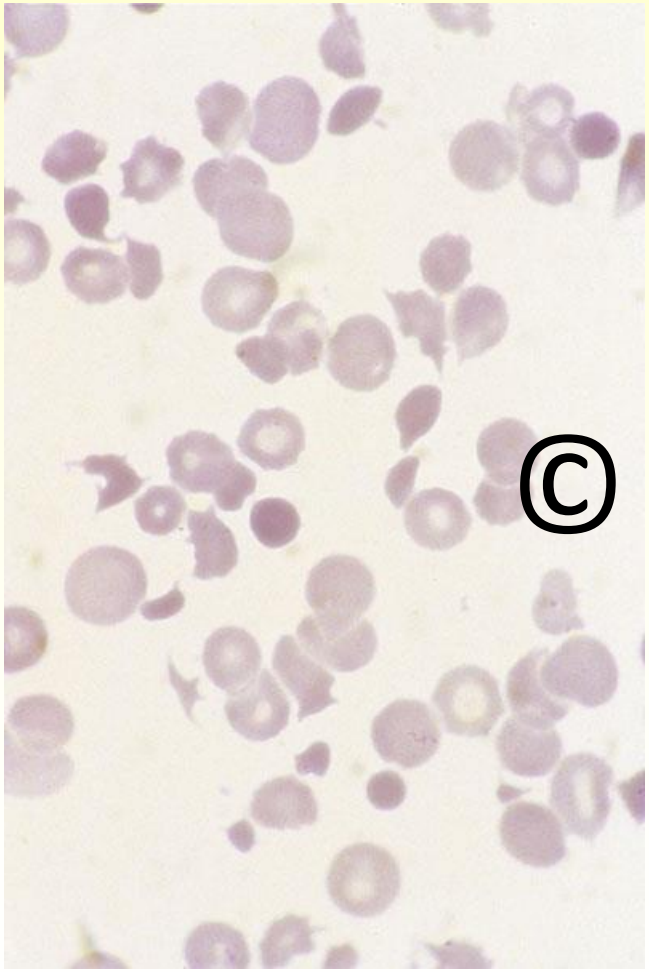
MCHC ↑or Hyperchromia flag (iii)

- Here there are two clues as to the cause of the hyperdense cells
- What is the likely explanation?



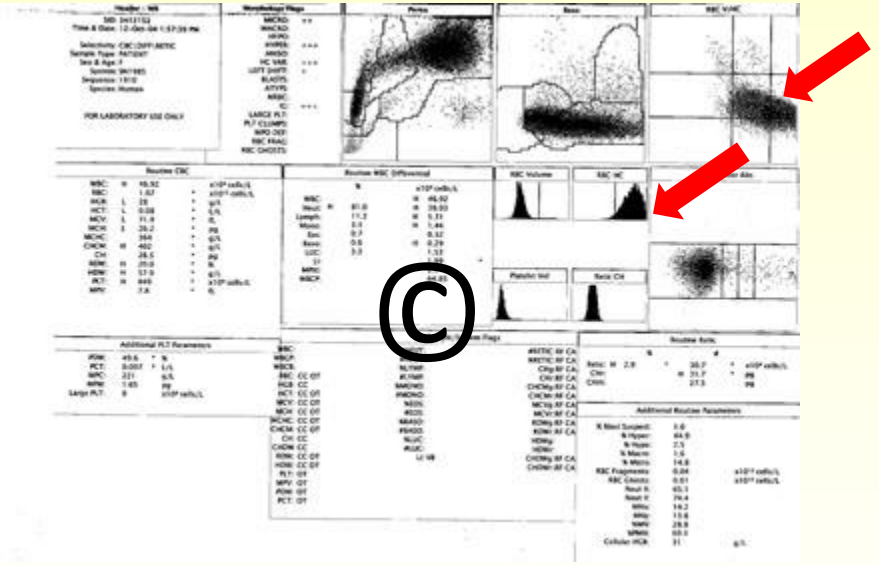
MCHC ↑ or Hyperchromia flag

- Here there are two clues as to the cause of the hyperdense cells
- What is the likely explanation?
- MAHA – HUS, TTP, HELLP, this example snakebite

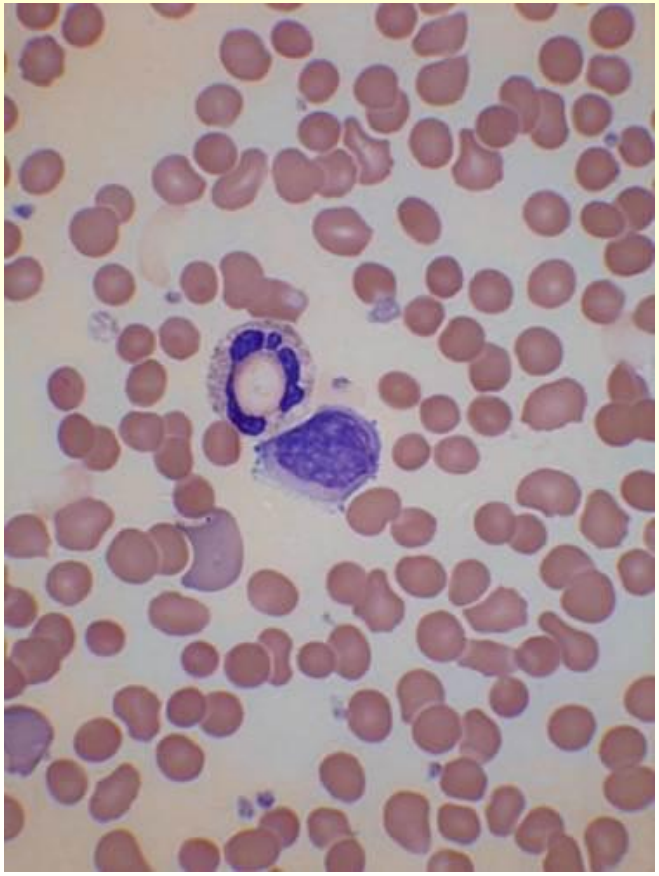


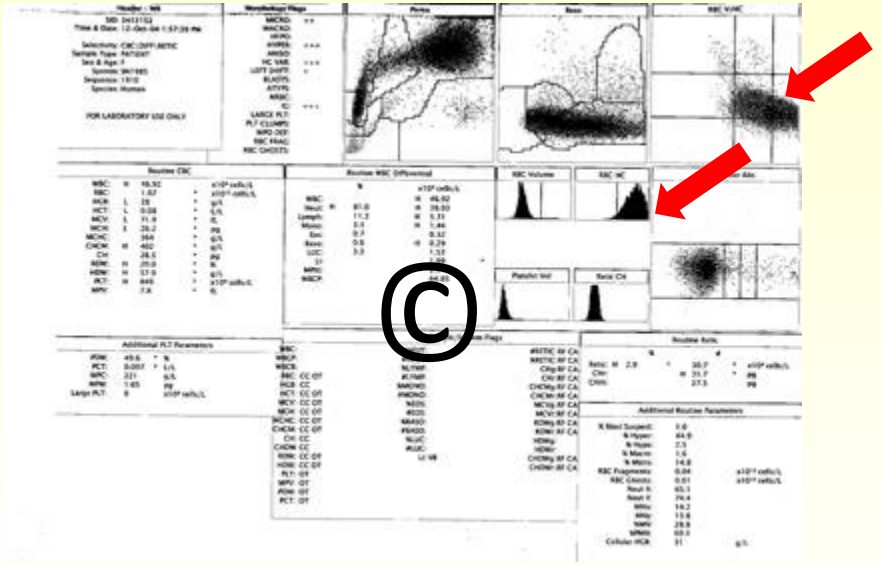
MCHC ↑or Hyperchromia flag (iv)

- What is the explanation in this child with a recent febrile illness?



MCHC ↑or Hyperchromia flag

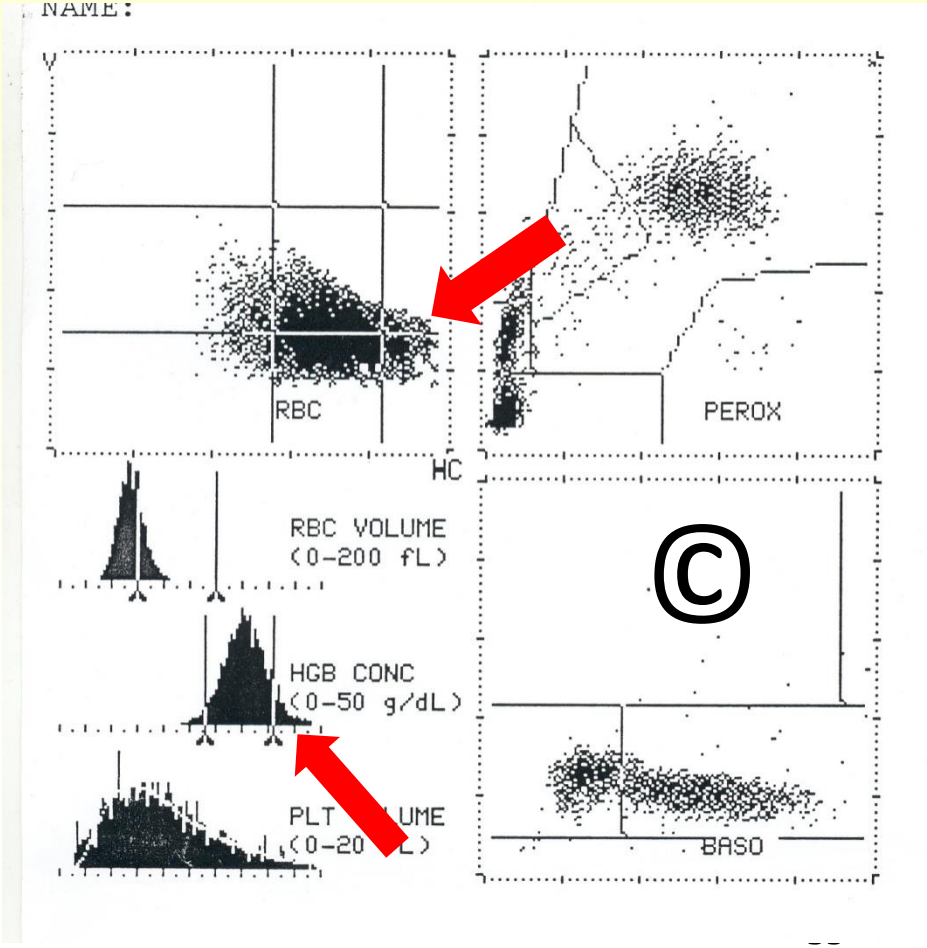




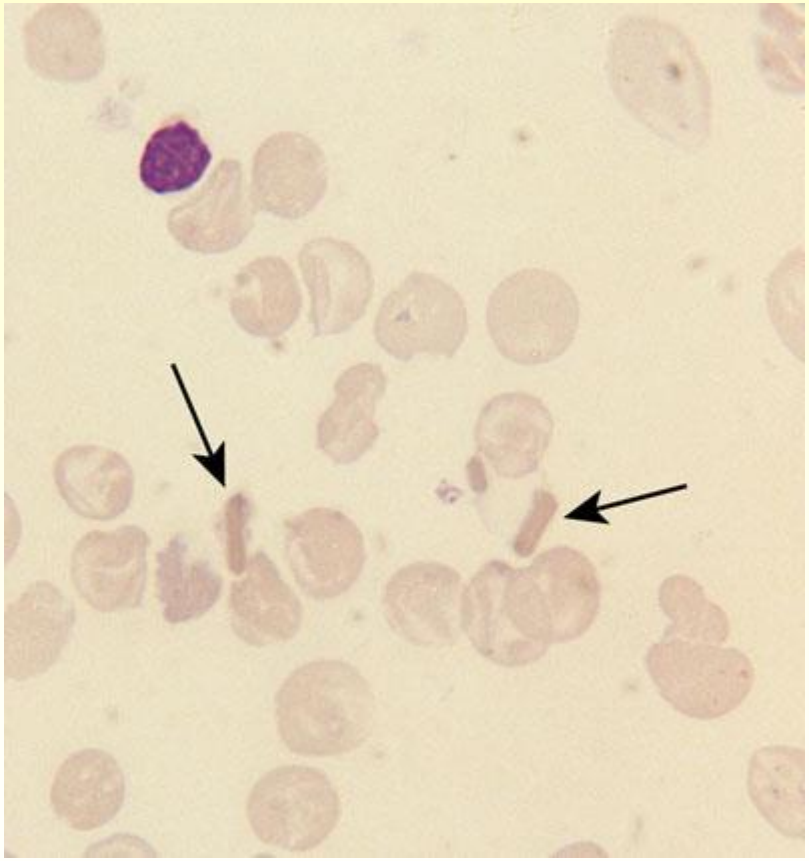
- Paroxysmal cold haemoglobinuria

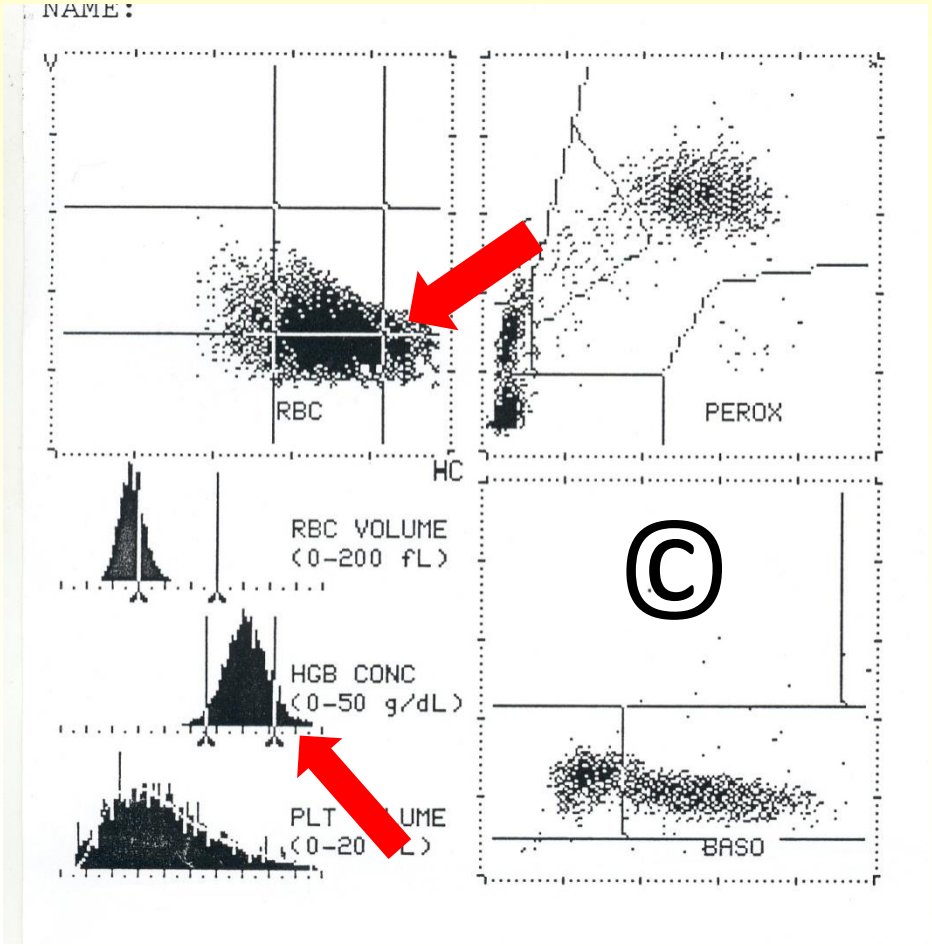
MCHC ↑ or Hyperchromia flag (v)

- What is the explanation this time (in an Afro-Caribbean woman)?



MCHC ↑ or Hyperchromia flag

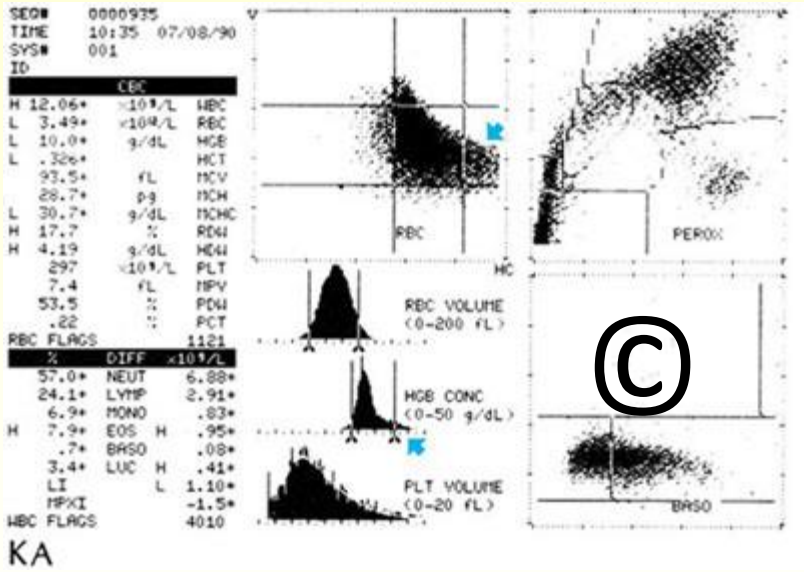




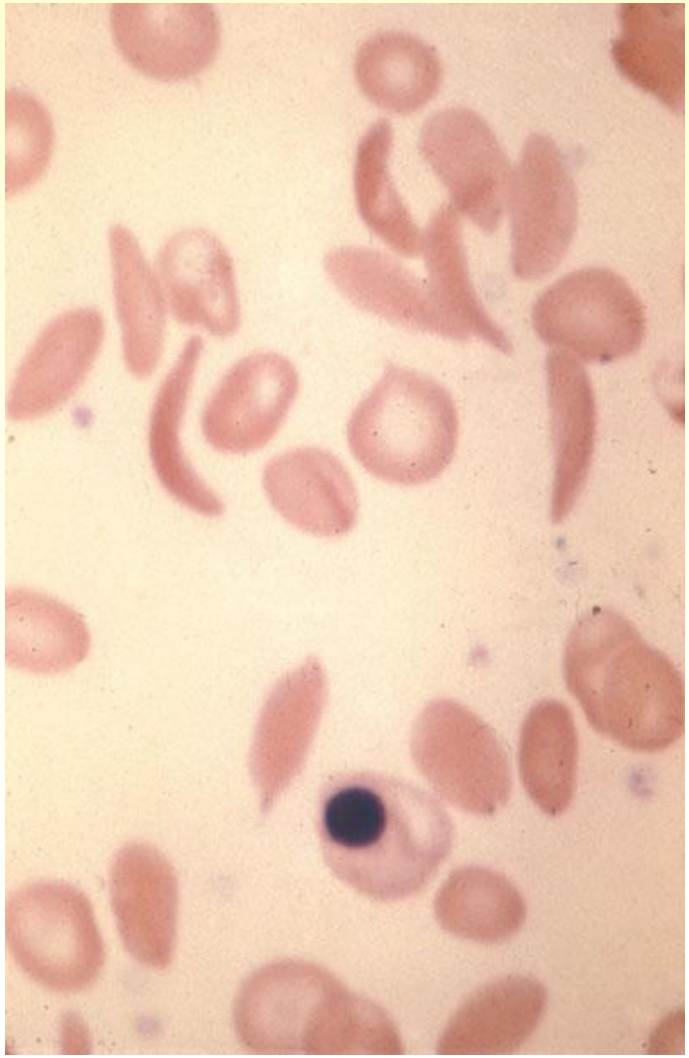
- Haemoglobin C/β^0 thalassaemia

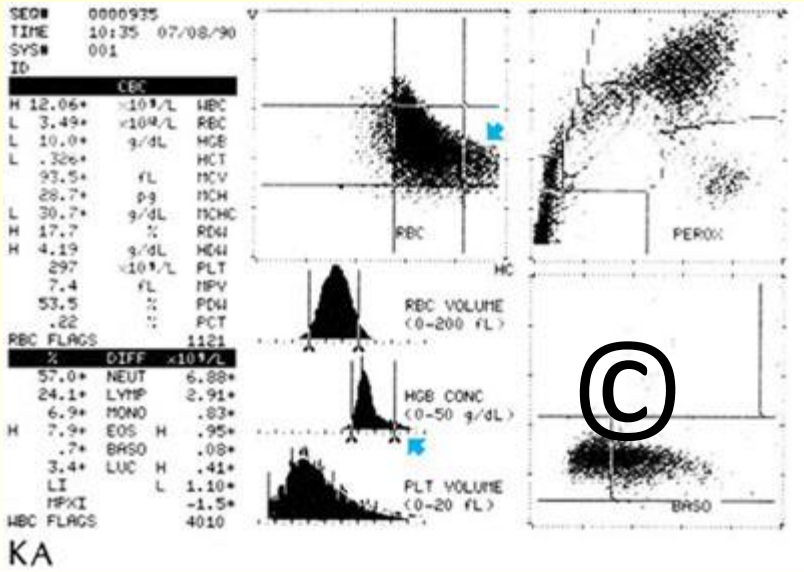
MCHC ↑ or Hyperchromia flag (vi)

- What is the explanation this time in an Afro-Caribbean man?



MCHC ↑ or Hyperchromia flag





Sickle cell anaemia

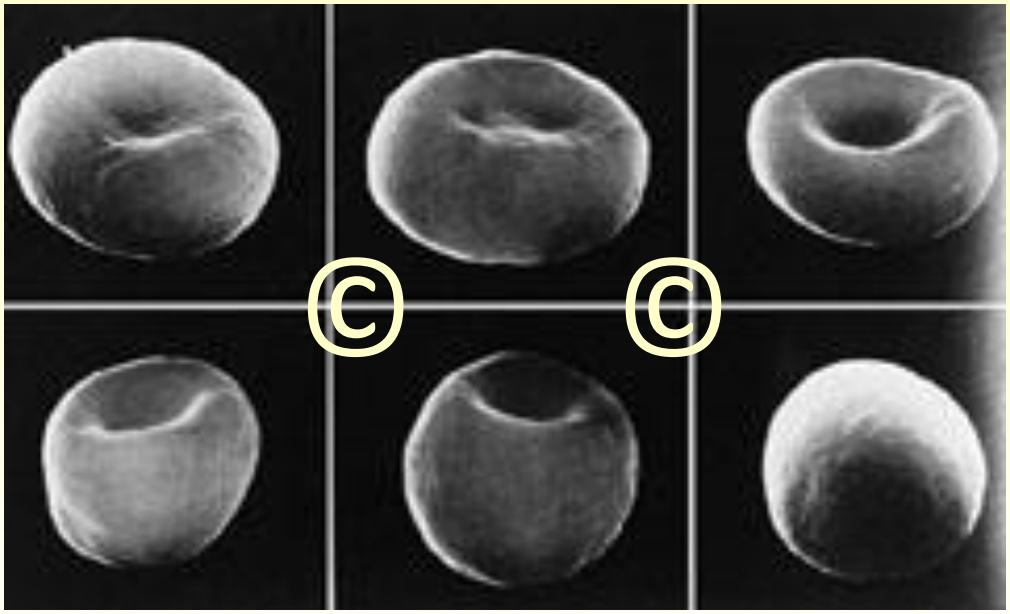
Providing a differential diagnosis

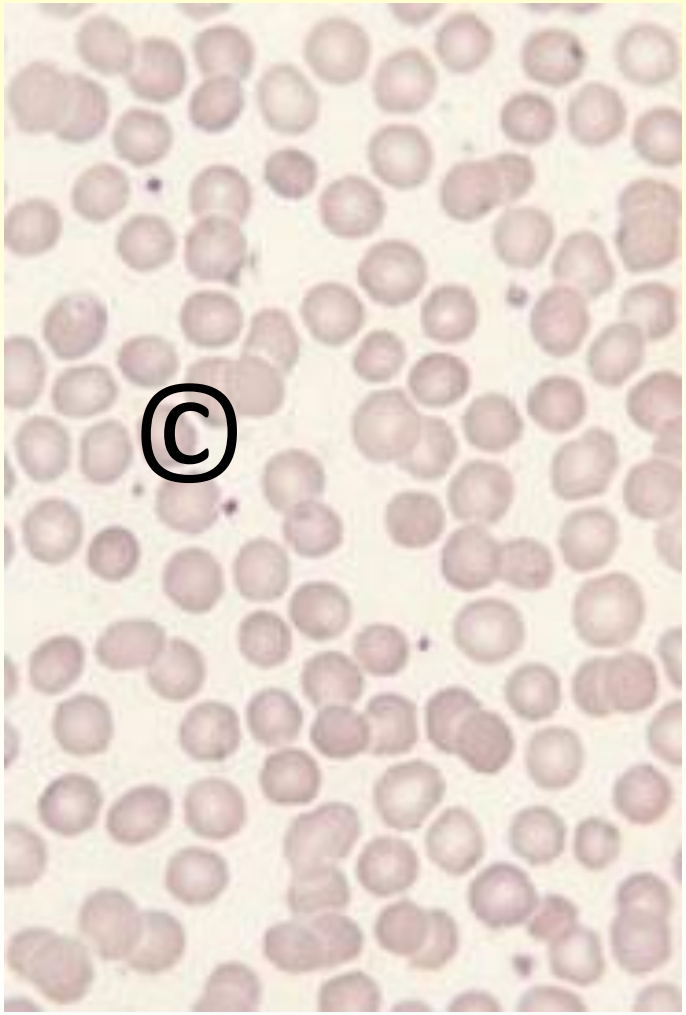
Providing a differential diagnosis

There are spherocytes. What could it be?

- Hereditary spherocytosis
- Immune haemolytic anaemia
  - Autoimmune
  - Alloimmune (neonatal, post-transfusion, anti-D, high dose IV Ig)
  - Drug-induced immune

Hereditary spherocytosis





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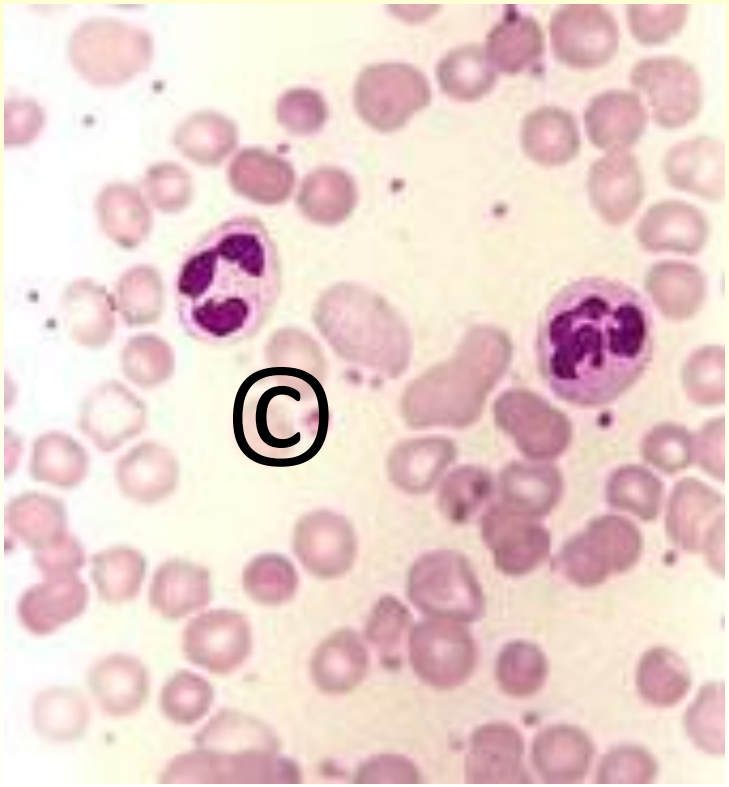
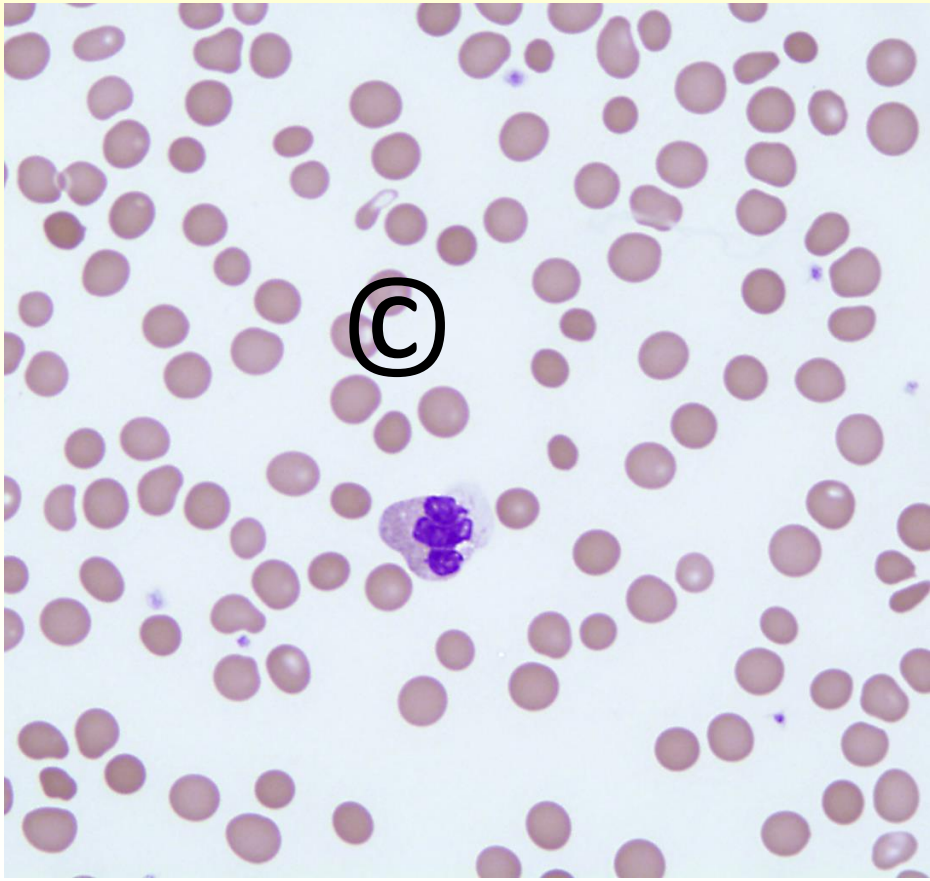
Autoimmune haemolytic anaemia



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

Acute anaemia with spherocytosis – why?

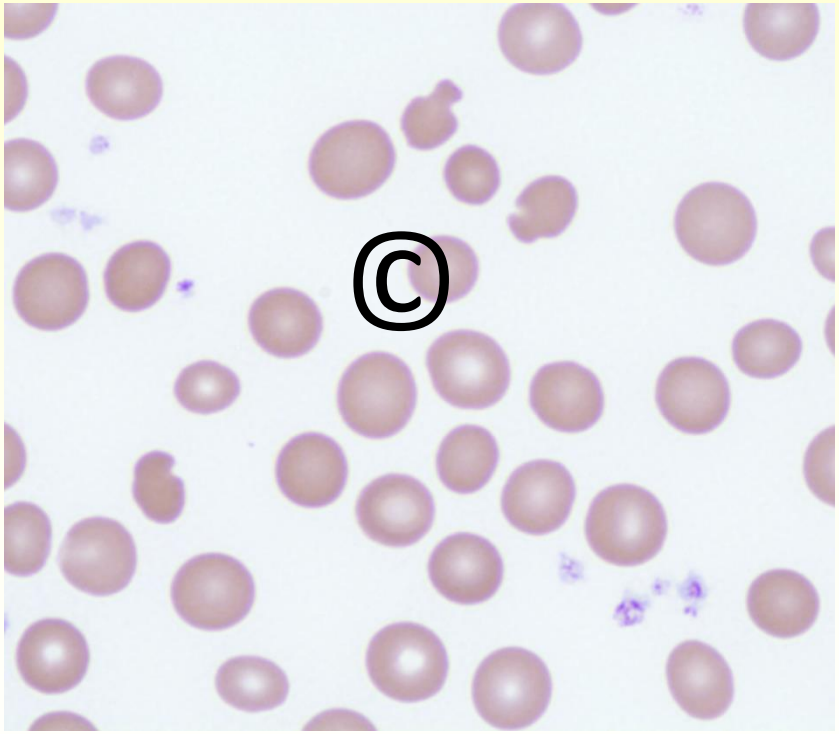
- A 7-year-old boy
- Rash, fever and pallor
- He has a history of neonatal jaundice
- Hb 30 g/l, reticulocytes 11 $\times 10^9$/l
- What is the problem?



From Leach M, Drummond M, Doig A, McKay P, Jackson R and Bain BJ, *Practical Flow Cytometry in Haematology: 100 worked examples*, Wiley, 2015.

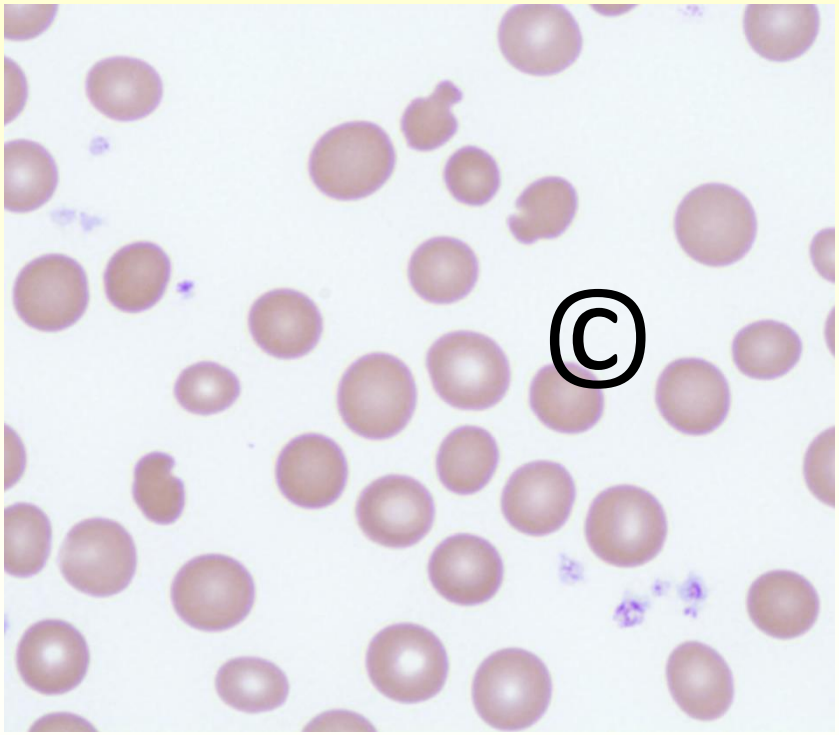
Hereditary spherocytosis plus parvovirus B19 infection

- What is the problem?
- **Previously undiagnosed hereditary spherocytosis with parvovirus B19 infection**

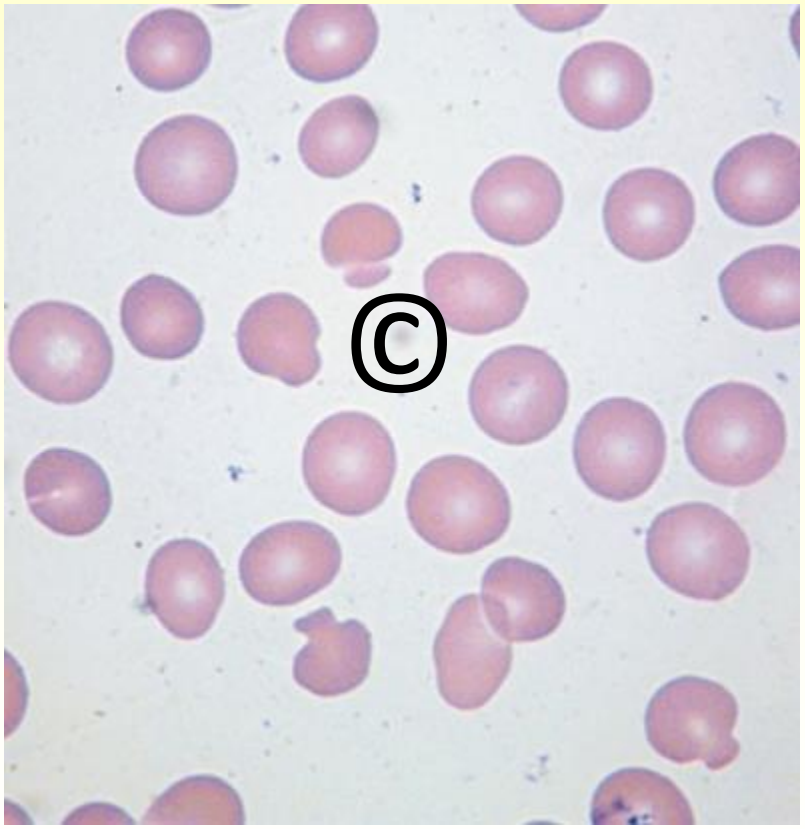


From Leach M, Drummond M, Doig A, McKay P, Jackson R and Bain BJ, *Practical Flow Cytometry in Haematology: 100 worked examples*, Wiley, 2015.

Hereditary spherocytosis – can you predict the genetic defect?



This case

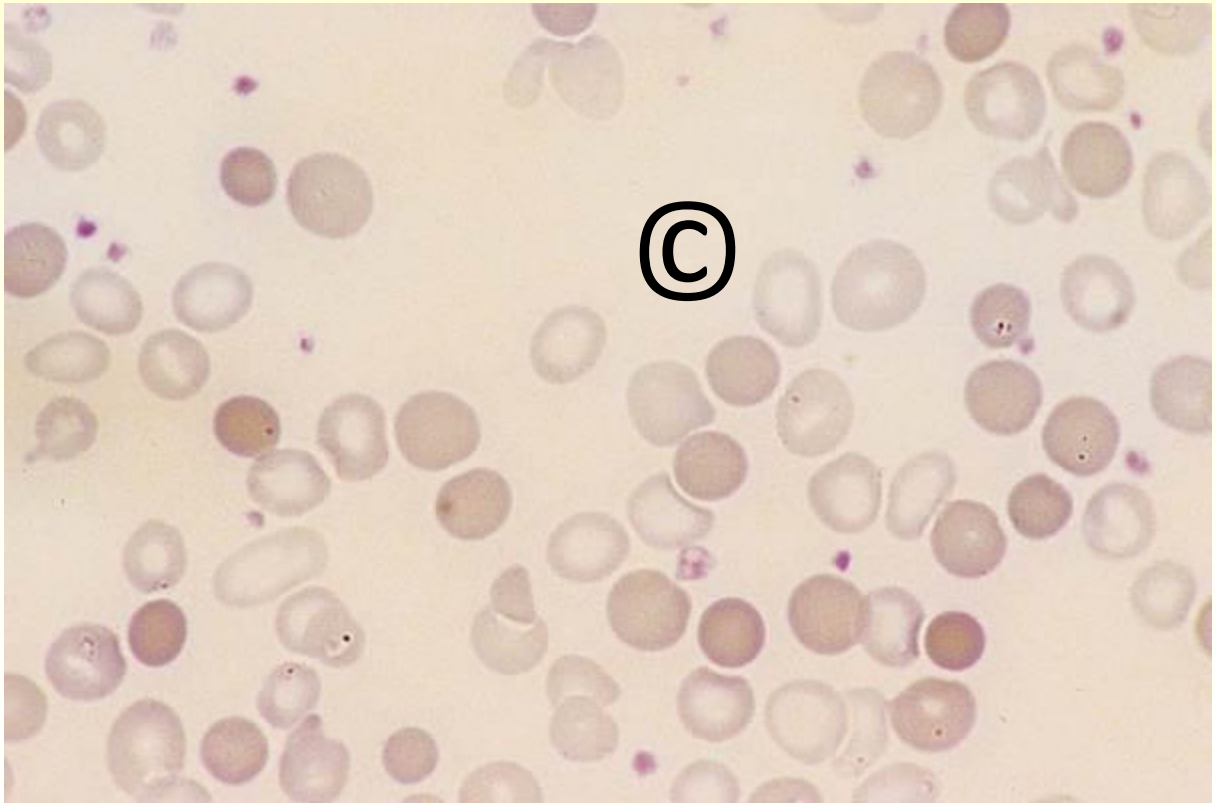


Band 3 deficiency

Leach et al., *Practical Flow Cytometry in Haematology: 100 worked examples*, Wiley, 2015.

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

This patient was transfused at the weekend – why are there spherocytes?



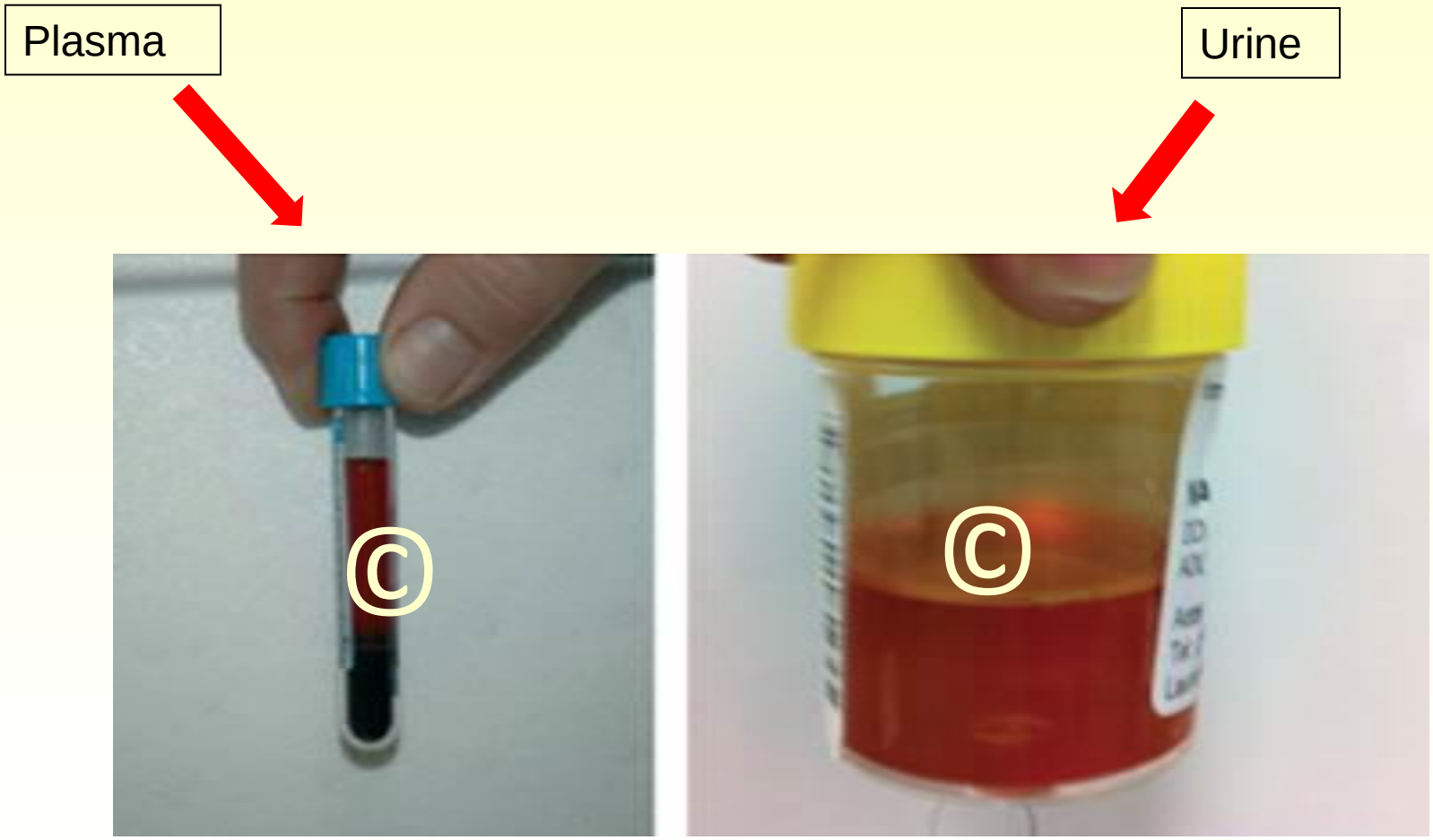
Bain BJ, 2014, Interactive Imagebank, 2nd Edn, Wiley-Blackwell

An unexpected acute spherocytic anaemia

- A woman with a diagnosis of autoimmune thrombocytopenia purpura presented with haemorrhage and a platelet count of 15 $\times 10^9$/l
- She was given high dose intravenous immunoglobulin and this was repeated 48 hours later
- Subsequently she noted red urine

Mohamed, Bates and Eastley (2013) *Br J Haematol*, **160**, 570.

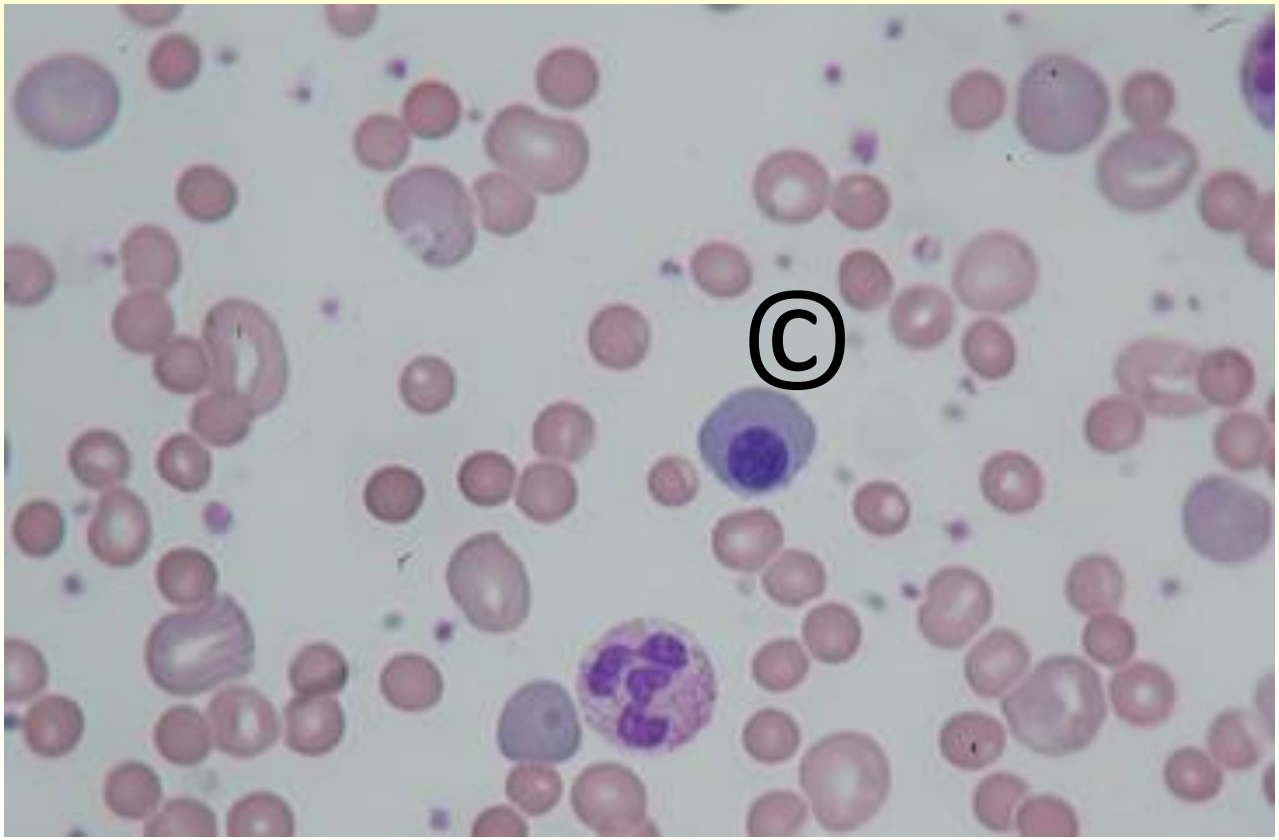
Acute spherocytic anaemia



Mohamed, Bates and Eastley (2013) *Br J Haematol*, **160**, 570.

Acute spherocytic anaemia

- What is the diagnosis?



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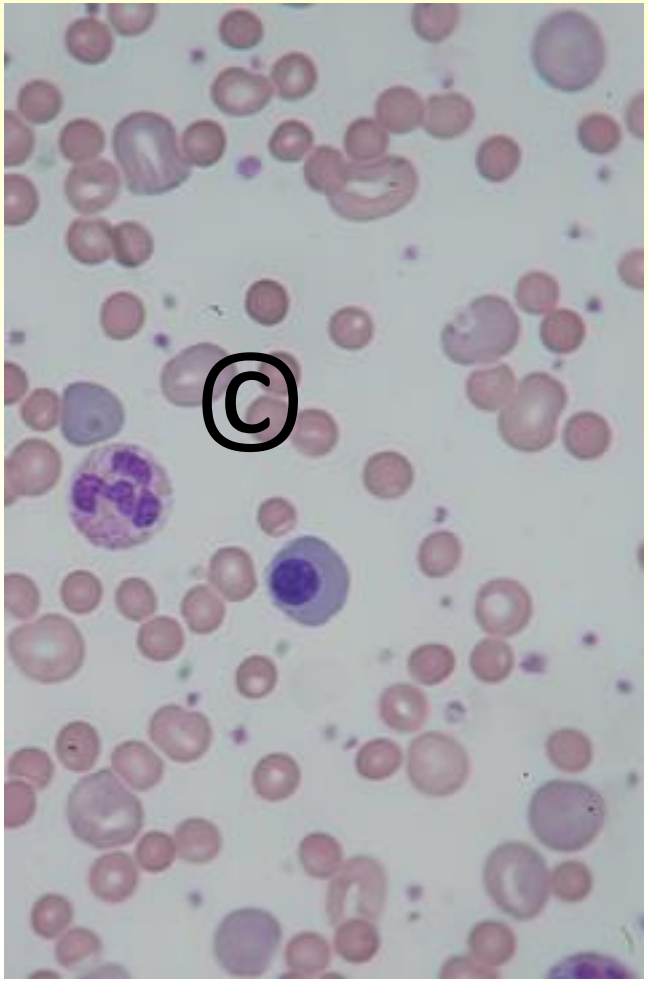
Acute spherocytic anaemia

- What is the diagnosis?
- Alloimmune haemolytic anaemia



Mohamed, Bates and Eastley (2013) *Br J Haematol*, **160**, 570.

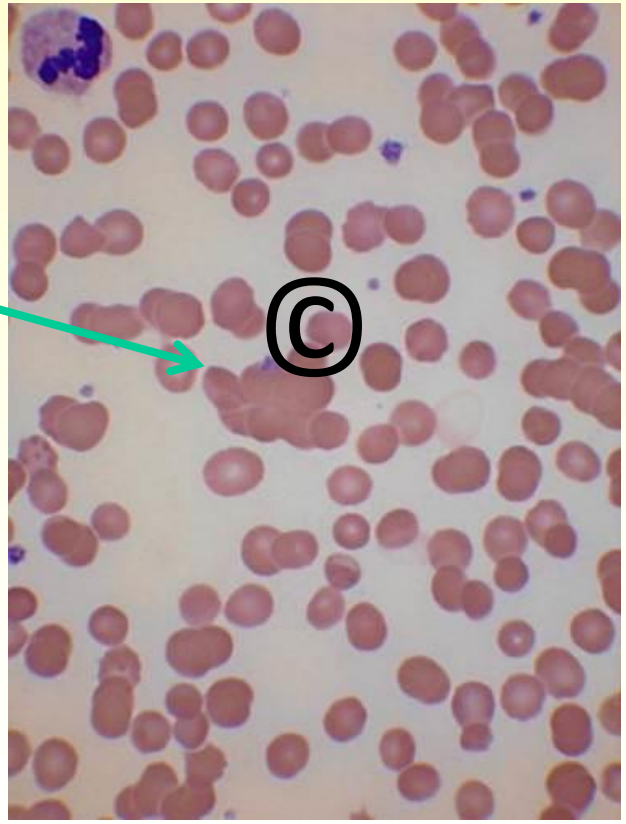
Acute spherocytic anaemia

- Haemoglobinuria
- Acute intravascular haemolysis due to anti-A in the immunoglobulin
- Direct antiglobulin test (Coombs test) positive
- Anti-A was eluted from the red cells
- Hb had fallen from122 to 80 g/l

Mohamed, Bates and Eastley (2013) *Br J Haematol*, **160**, 570.

Paroxysmal cold haemoglobinuria – spherocytosis plus other features

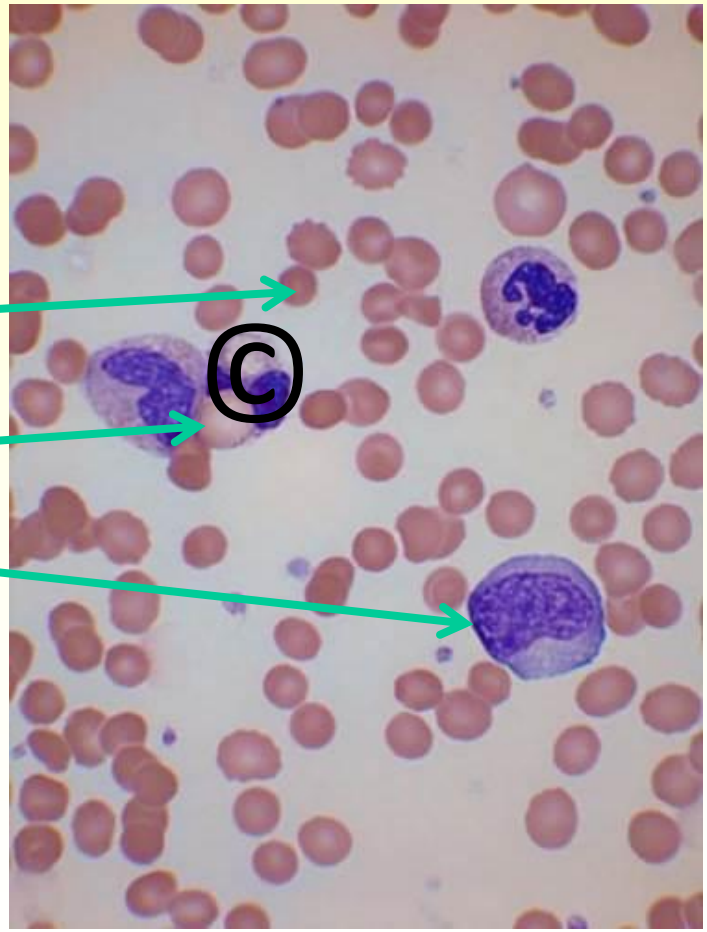
- The diagnosis can be made from the blood film
  - Agglutination
  - Spherocytes
  - Erythrophagocytosis
  - Atypical lymphocytes



Bharadwaj V, Chakravorty S and Bain BJ (2011) The cause of sudden anemia revealed by the blood film. *Am J Hematol*, **87**, 520.

Paroxysmal cold haemoglobinuria – spherocytosis plus other features

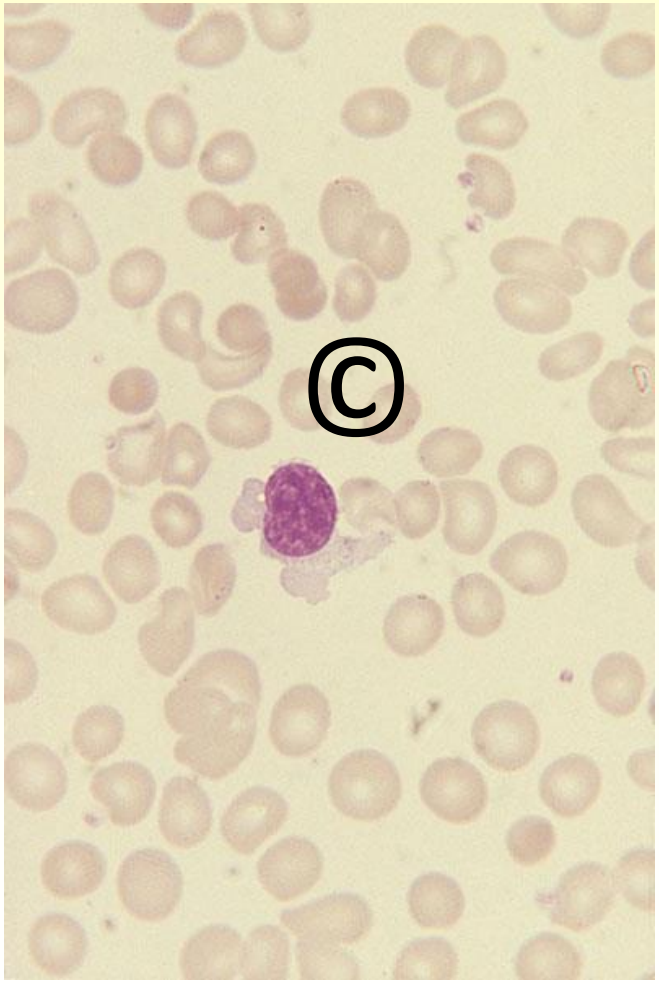
- The diagnosis can be made from the blood film
  - Agglutination
  - Spherocytes
  - Erythrophagocytosis
  - Atypical lymphocytes



Bharadwaj V, Chakravorty S and Bain BJ (2011) The cause of sudden anemia revealed by the blood film. *Am J Hematol*, **87**, 520.

Acute cold antibody induced haemolytic anaemia

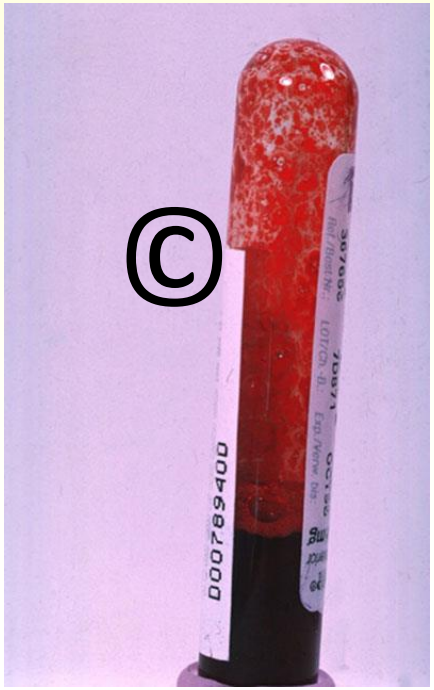
- This case associated with infectious mononucleosis
- Anti-i detected

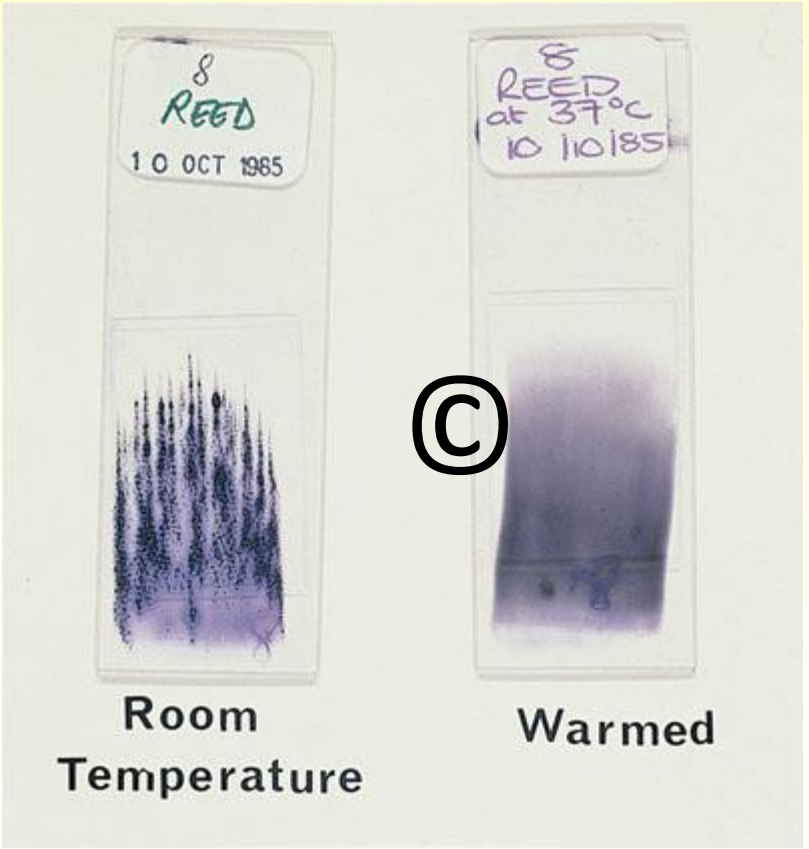


Bain BJ, 2014, Interactive Imagebank, 2nd Edn, Wiley-Blackwell

Macroscopy as well as microscopy!

- Chronic cold haemagglutinin disease

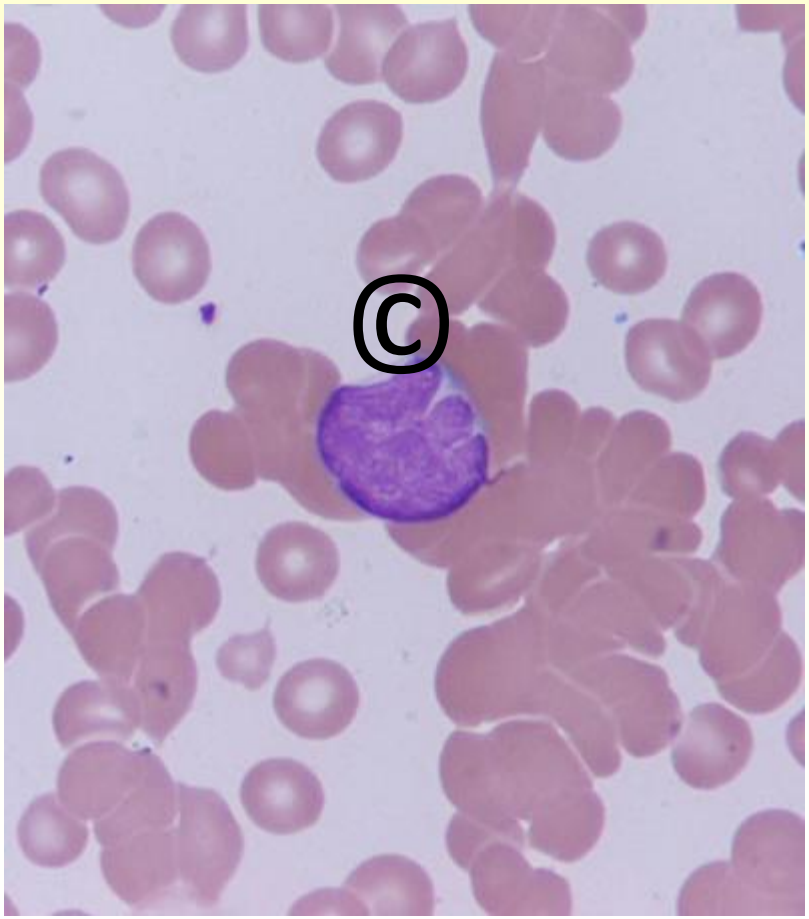




Bain BJ, 2014, Interactive Imagebank, 2nd Edn, Wiley-Blackwell

Chronic cold haemagglutinin disease

- The underlying cause is apparent

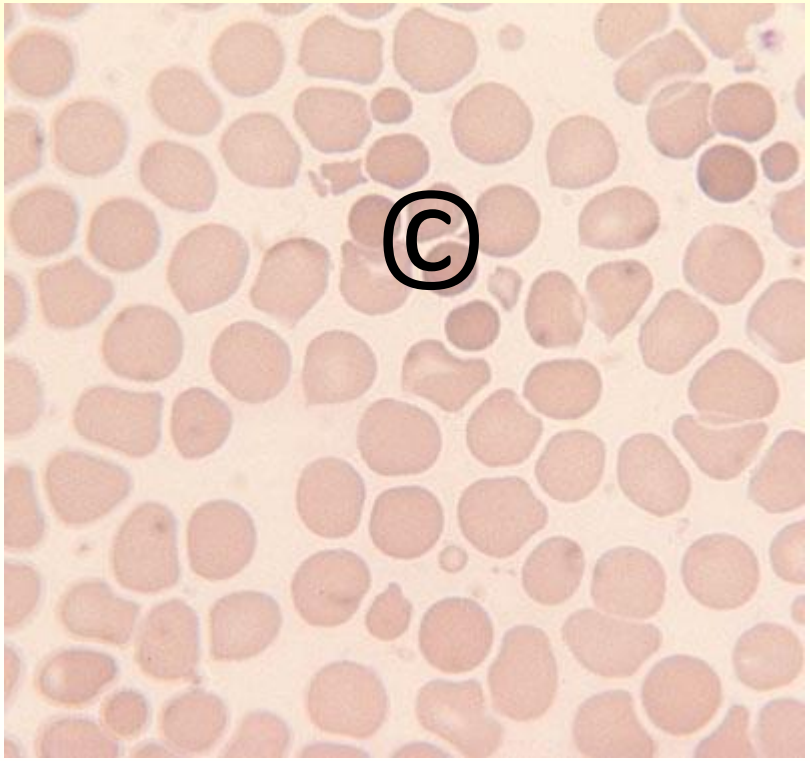


With thanks to Dr Abbas Hashim Abdulsalam, Baghdad

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

You should not need a blood film to recognise the cause of these spherocytes

- What is the cause?



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

Contributing to the diagnosis

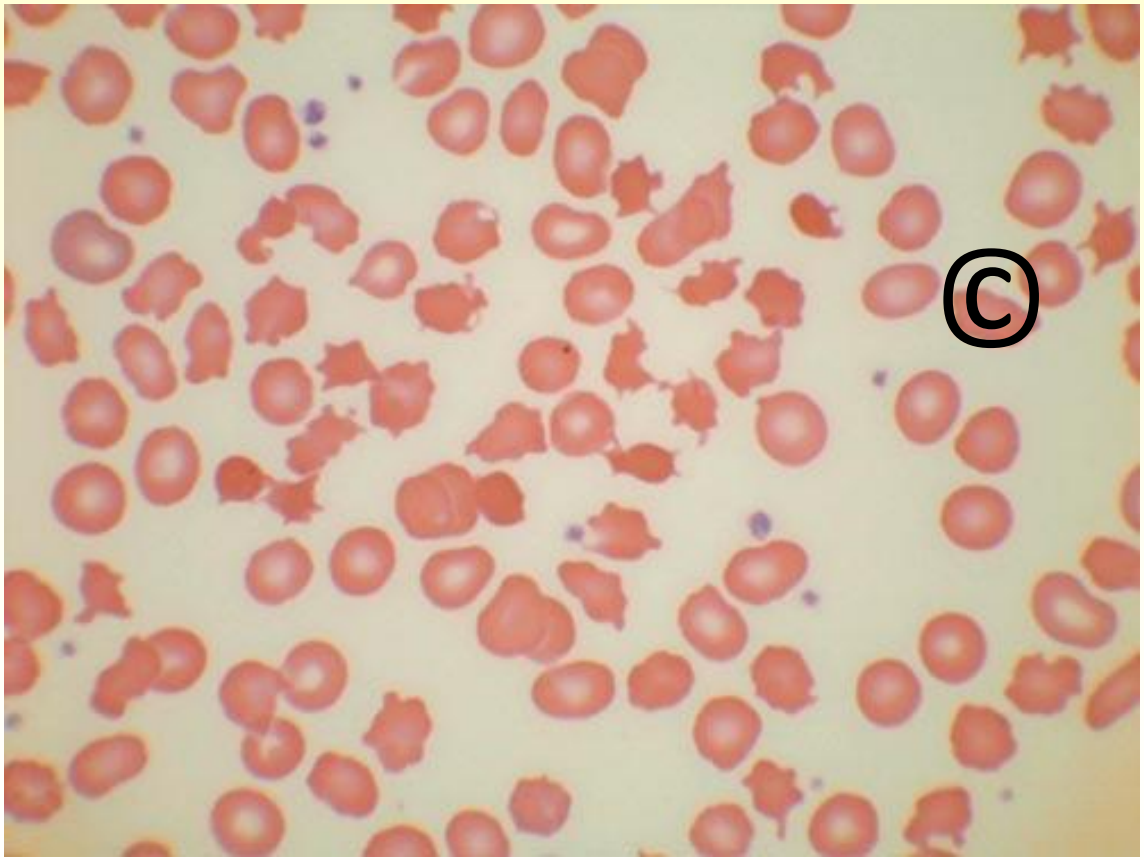
Choreoacanthocytosis

- A 34-year-old woman of Pakistani origin
- Her parents were first cousins
- Developed dysphagia, dysarthria, a stutter, grimacing and facial tics (blinking and twitching of eyes, protrusion of tongue)
- 4 years later developed chorea and a blood count and film were done
- Her blood count was normal

Bain BJ and Bain PG (2013) Choreo-acanthocytosis. *Am J Hematol*, **88**, 712.

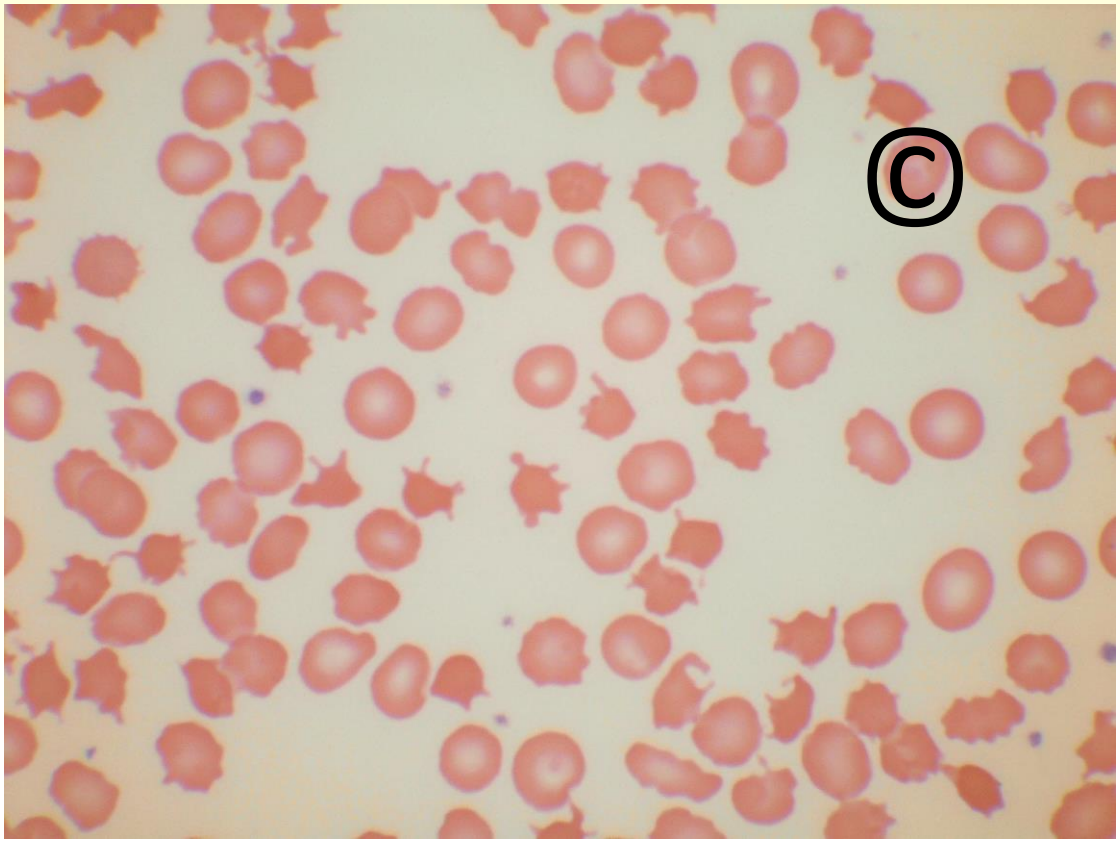
Contributing to the diagnosis

Choreoacanthocytosis



Contributing to the diagnosis

The diagnosis was further confirmed by showing reduced red cell chorein

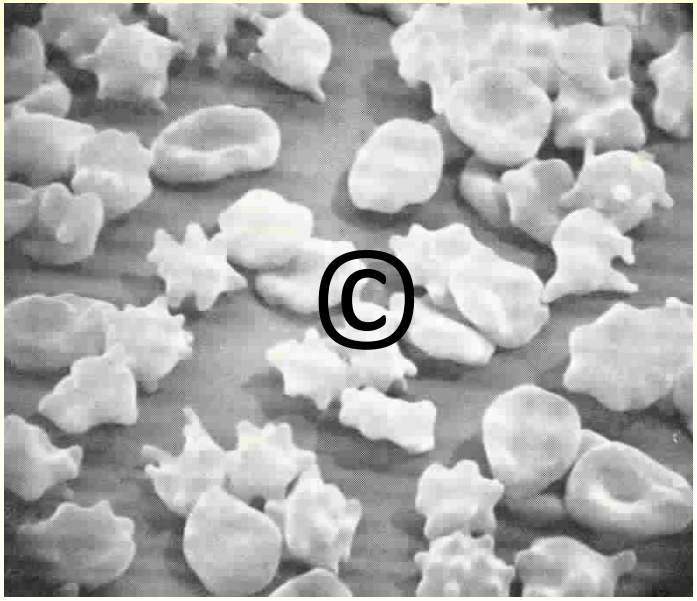


Contributing to the diagnosis

Four types of neuroacanthocytosis

Syndrome	**Mutated gene and inheritance**	**Clinicopathological features**
Choreoacanthocytosis	*VPS13A*, autosomal recessive	Adult onset progressive neurodegeneration, myopathy, often epilepsy
McLeod phenotype	*KX*, X-linked recessive	Adult onset progressive neurodegeneration, myopathy, cardiomyopathy, weak or absent expression of Kell antigens
Huntingdon-like disease 2 (some cases)	*JPH3*, autosomal dominant	Adult onset progressive neurodegeneration
Pantothenate-kinase associated neurodegeneration (some cases)	*PANK2*, autosomal recessive	Childhood onset progressive neurodegeneration, pallidal degeneration, sometimes retinitis pigmentosa

The McLeod phenotype, another type of neuroacanthocytosis



Symmans et al (1979) *Br J Haematol*, **42**, 575.

Emergency red cell diagnosis

Microangiopathic haemolytic anaemia – differential diagnosis

- Haemolytic uraemic syndrome
- Thrombotic thrombocytopenic purpura
- Atypical haemolytic uraemic syndrome (defects of complement pathway)
- HELLP
- Drug-induced MAHA
- Post-transplant MAHA

Not forgetting mechanical haemolytic anaemia

A 17-year-old woman presents with anaemia and hypertension. Her blood film shows fragments and polychromasia. She is found to have a creatinine of 285 μmol/l, platelet count 40 × 10^9/l, and ADAMTS13 of 55% (NR ≥ 70%)

The most likely diagnosis is

- Atypical haemolytic uraemic syndrome
- Haemolytic uraemic syndrome (HUS)
- HELLP syndrome
- Malignant hypertension
- Thrombotic thrombocytopenic purpura

Modified from Bain BJ (2016) *Multiple Choice Questions for Haematology and Core Medical Trainees*, Wiley-Blackwell.

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- HELLP syndrome
- Malignant hypertension
- Thrombotic thrombocytopenic purpura

Modified from Bain BJ (2016) *Multiple Choice Questions for Haematology and Core Medical Trainees*, Wiley-Blackwell.

Why is this the most likely diagnosis?

- The ADAMTS13 is not low enough to favour TTP
- TTP usually has platelet count <30 × 10^9/l and creatinine less than 220 μmol/l
- HUS does not usually have a platelet count as low as 40 × 10^9/l and there is no history of diarrhoea
- Atypical HUS can have severe thrombocytopenia

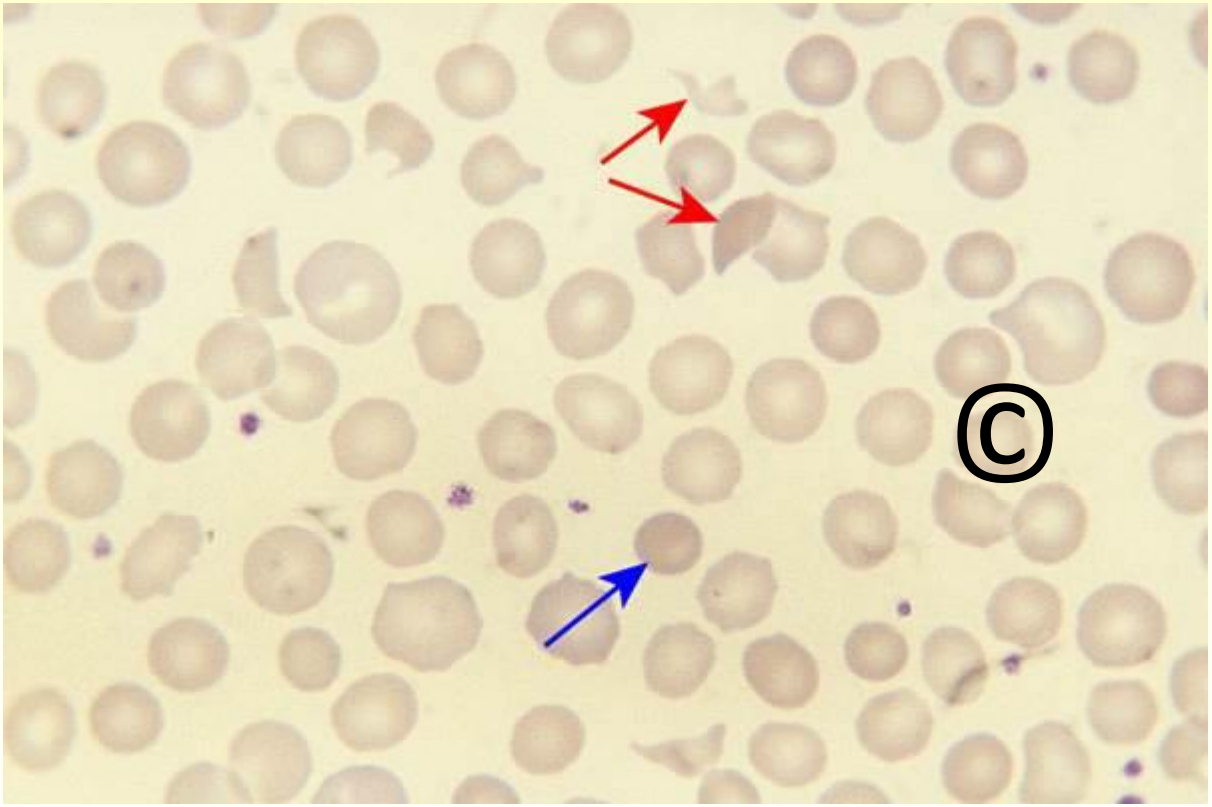
How would you manage the patient?

- In real life the ADAMTS13 assay results would not be immediately available
- In that case, how might you manage the patient?

How would you manage the patient?

- In real life the ADAMTS13 assay results would not be immediately available
- In that case, how might you manage the patient?
- Plasma exchange till assay results available then eculizumab
- Don’t forget the need for meningococcal vaccination

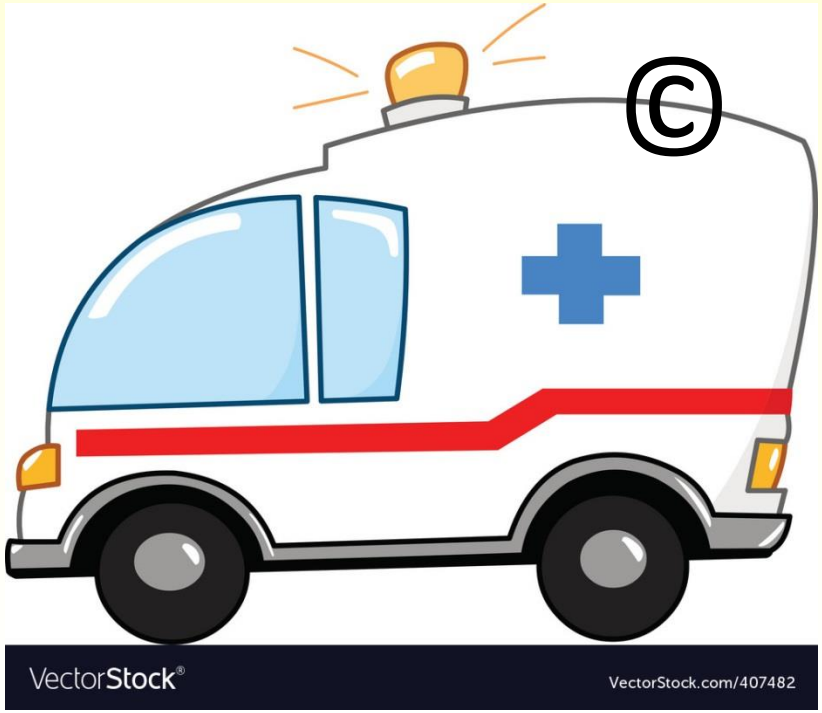
Haemolytic uraemic syndrome



- A blood film must **always** be done in any patient presenting with acute kidney injury

Thrombotic thrombocytopenic purpura

- This is an emergency



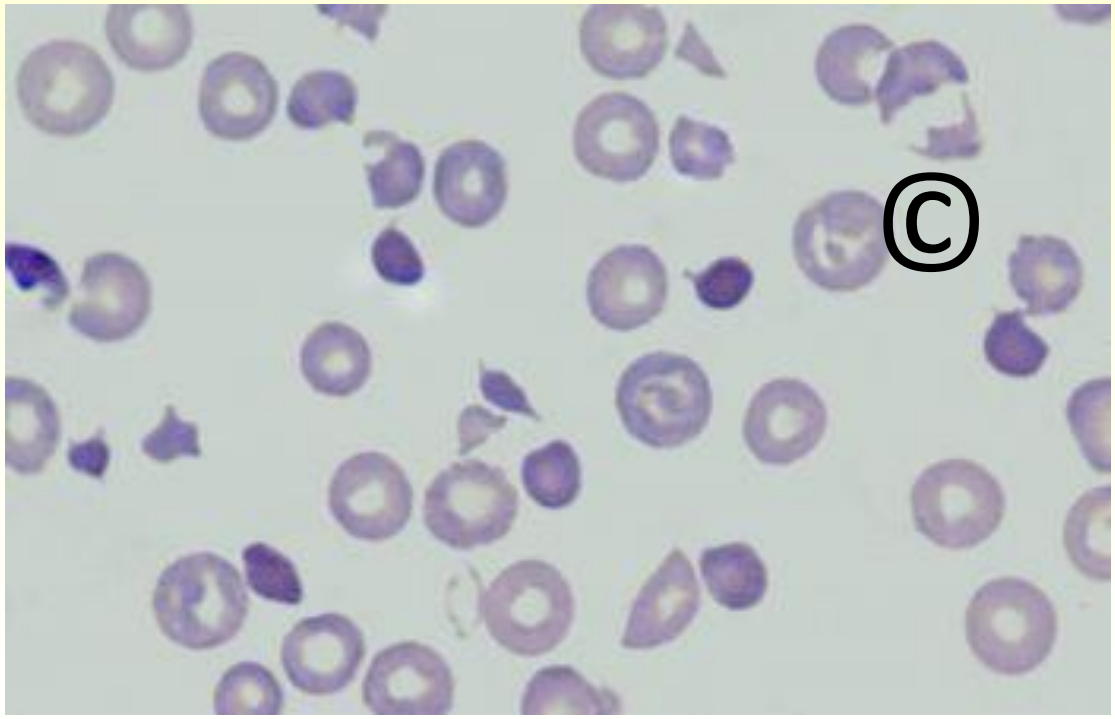
Thrombocytopenia – another genuine emergency

- 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

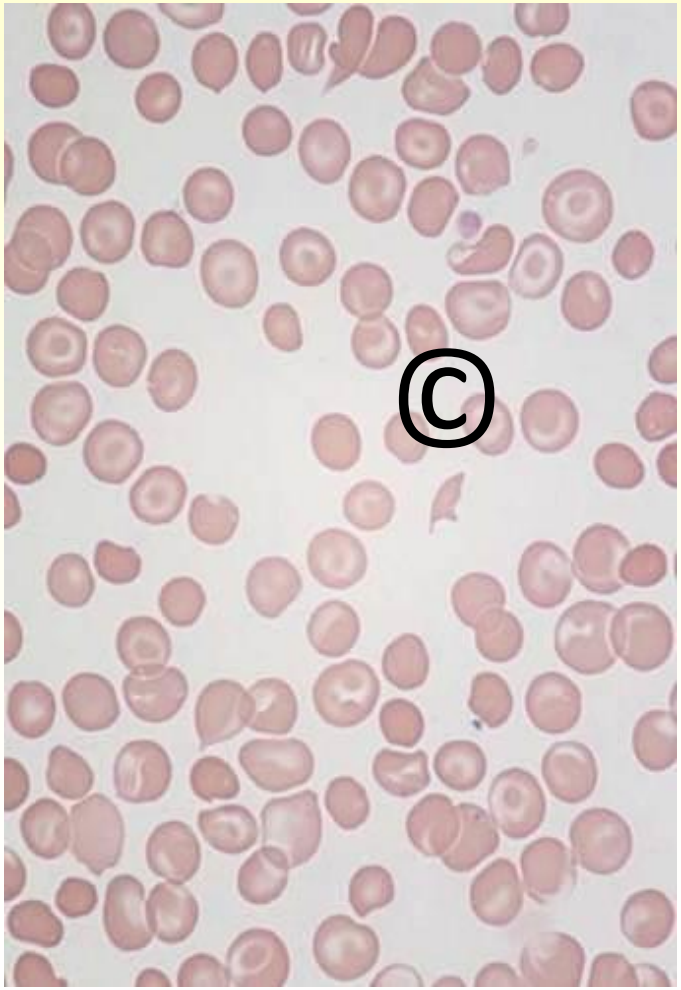
- The blood film is very important

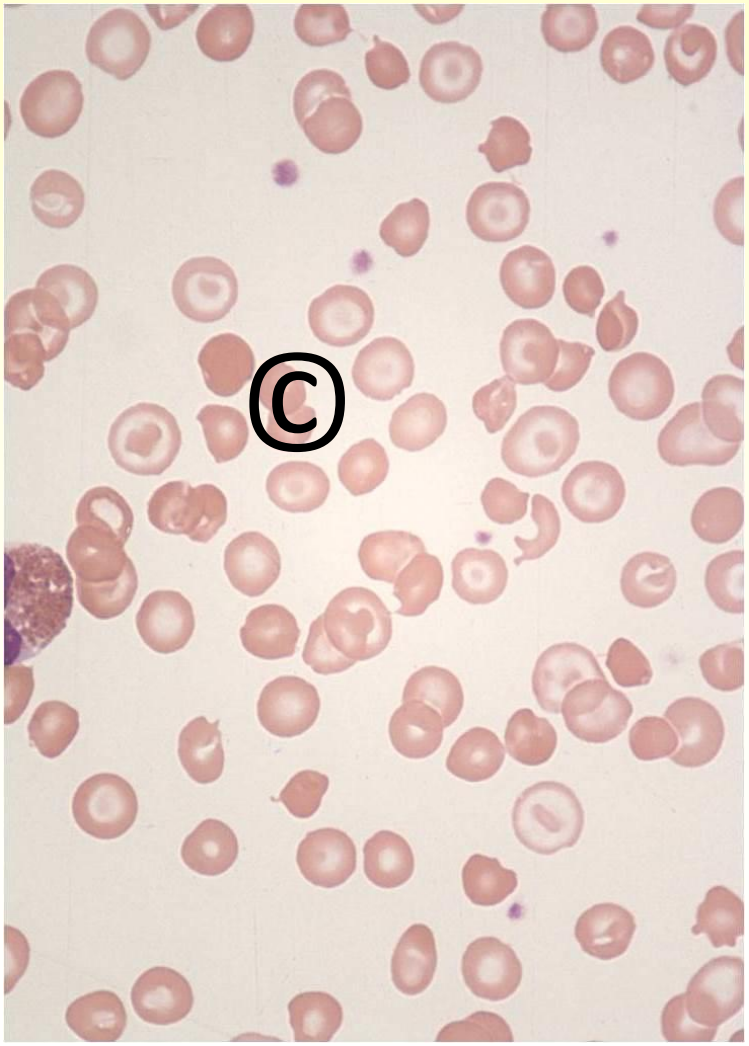


From Vallespi T and Garcia-Alonso L, Atlas of Blood cells and Blood Disease.
http://www.atlasbloodcells.es/

However … things are not always so simple

- Fragments can be rare





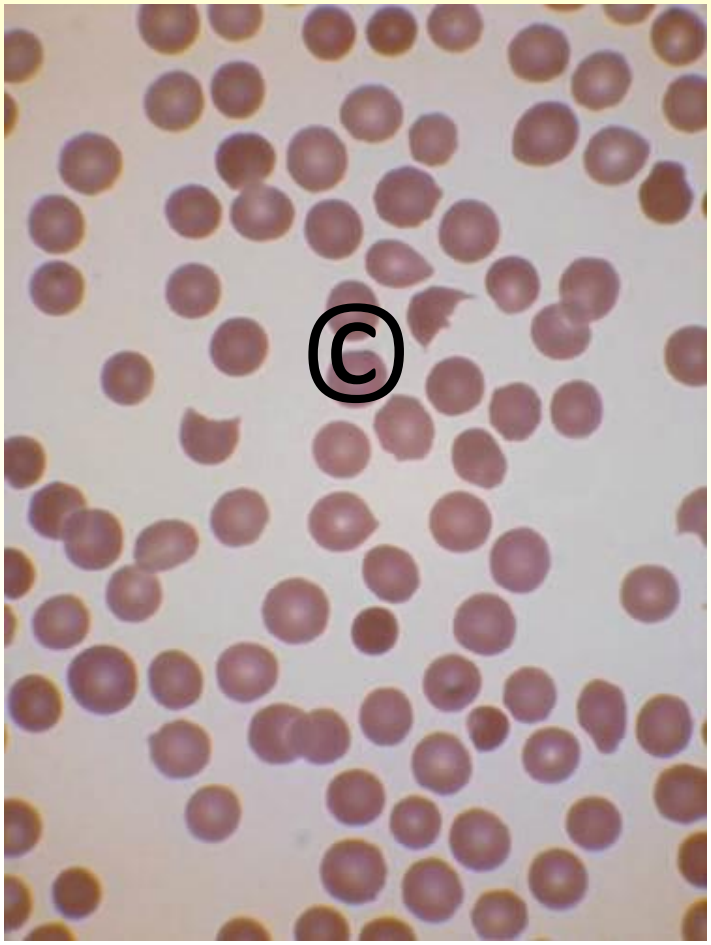
But … things are not always so simple

- This was a 26-year-old Indian woman was homozygous for haemoglobin E
- Hb 88 g/l (normally *c*. 96) with reticulocyte count 3.8% and platelet count <10 $\times 10^9$/l
- She was given three platelet concentrates
- The next morning she was confused and then lost consciousness
- ADAMTS13 <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.

HELLP –**H**aemolysis **E**levated **L**iver enzymes **L**ow **P**latelets

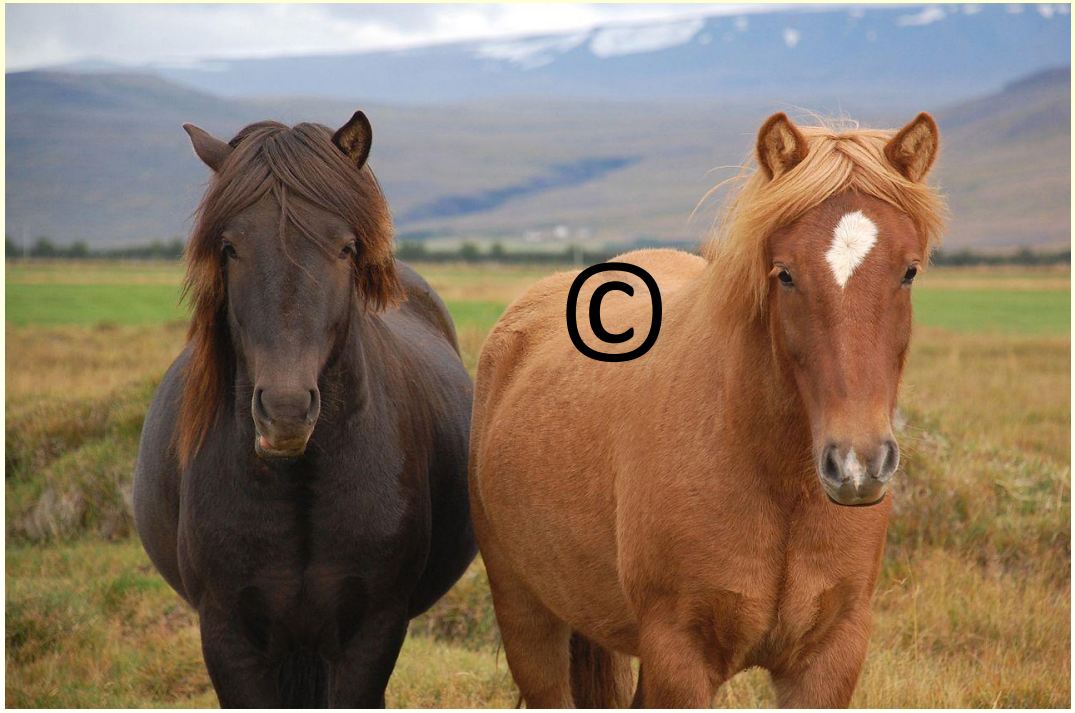
- A 31-year-old pregnant woman with hypertension and oedema
- Abnormal renal and hepatic function
- Anaemic
- Platelet count 25 $\times 10^9$/l



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

Not only horses …

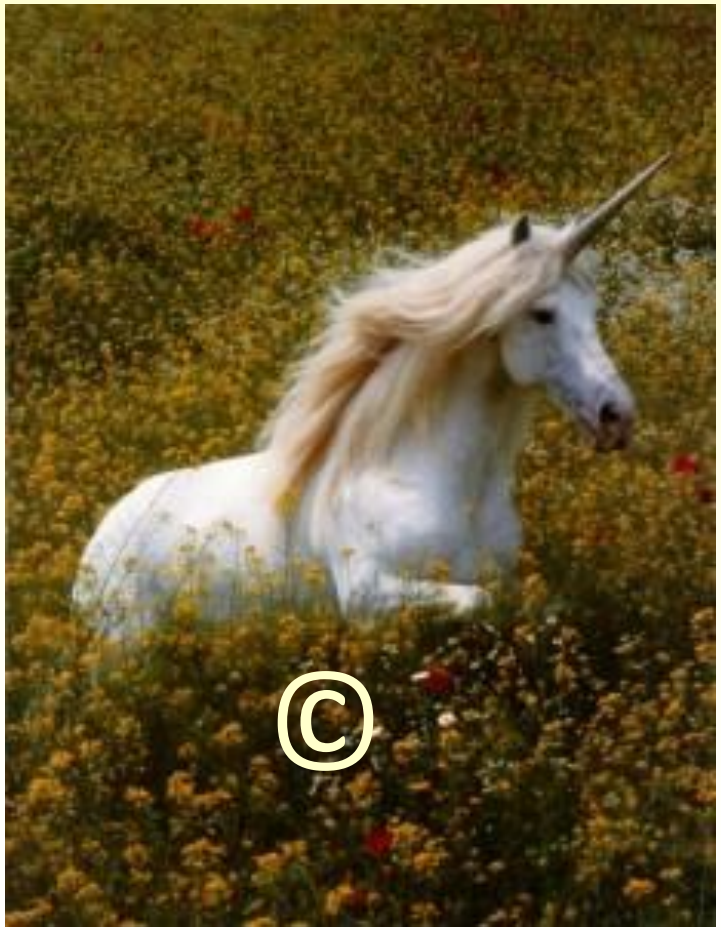
- If you hear the sound of hooves don’t think only of horses



http://en.wikipedia.org/wiki/Icelandic_horse

Not only horses …

- It may be something rarer



http://www.jeremynoeljohnson.com/insight/the-blacksmith-and-the-unicorn/

… not always so simple

- 34-year-old primigravida at 22 weeks gestation was admitted with fever (39.8° C), right abdominal discomfort, nausea, vomiting, and rapidly worsening haemolytic anaemia, severe thrombocytopenia, and raised concentrations of aspartate (AST) and alanine (ALT) aminotransferases
- Hb 101 g/l, falling to 93 g/l, platelets to 60×10^9/l, falling to 16×10^9/l
- Haptoglobin undetectable

… not always so simple

- Lactate dehydrogenase (LDH) 1579 iu/l
- Total bilirubin 71 μmol/l (normal 5–20) and direct bilirubin 45 μmol/l (0–9)
- D-dimers 1386 μg/l, rising to 9745 μg/l (0–278);
- Provisional diagnosis: HELLP syndrome
- What has gone wrong?

Tournoy et al. (2006) Haemolysis, elevated liver enzymes, and thrombocytopenia in a 34-year-old pregnant woman. *Lancet*, **368**, 90.

… not always so simple

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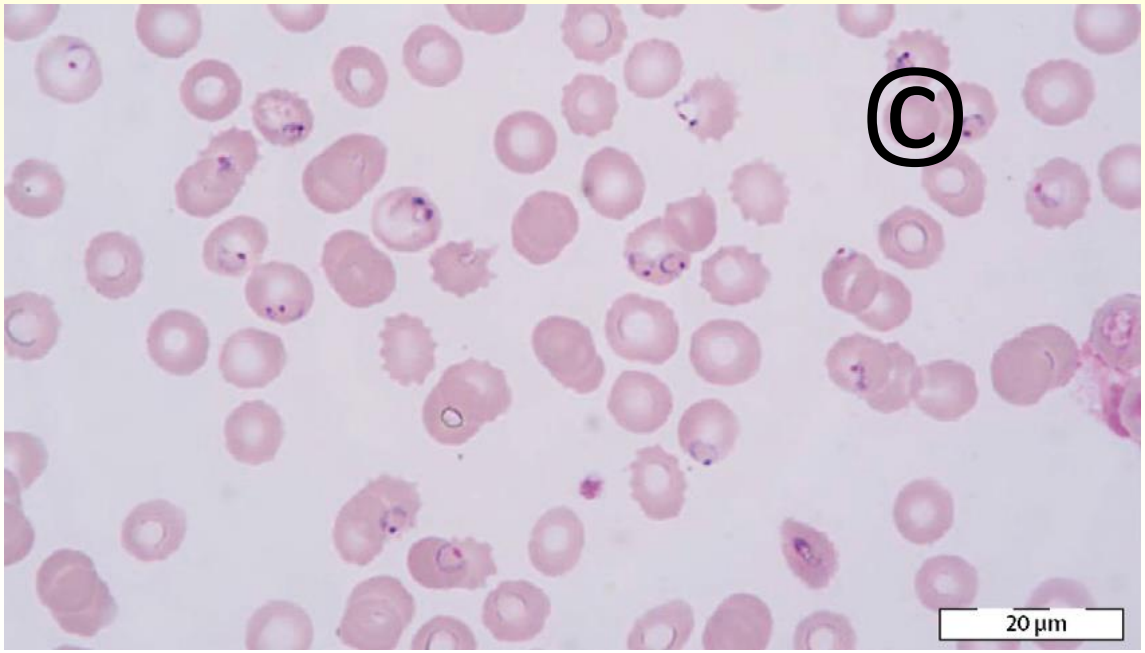
- Provisional diagnosis: HELLP syndrome
- What has gone wrong?
  - Clinical history
  - Blood film

Tournoy et al. (2006) Haemolysis, elevated liver enzymes, and thrombocytopenia in a 34-year-old pregnant woman. *Lancet*, **368**, 90.

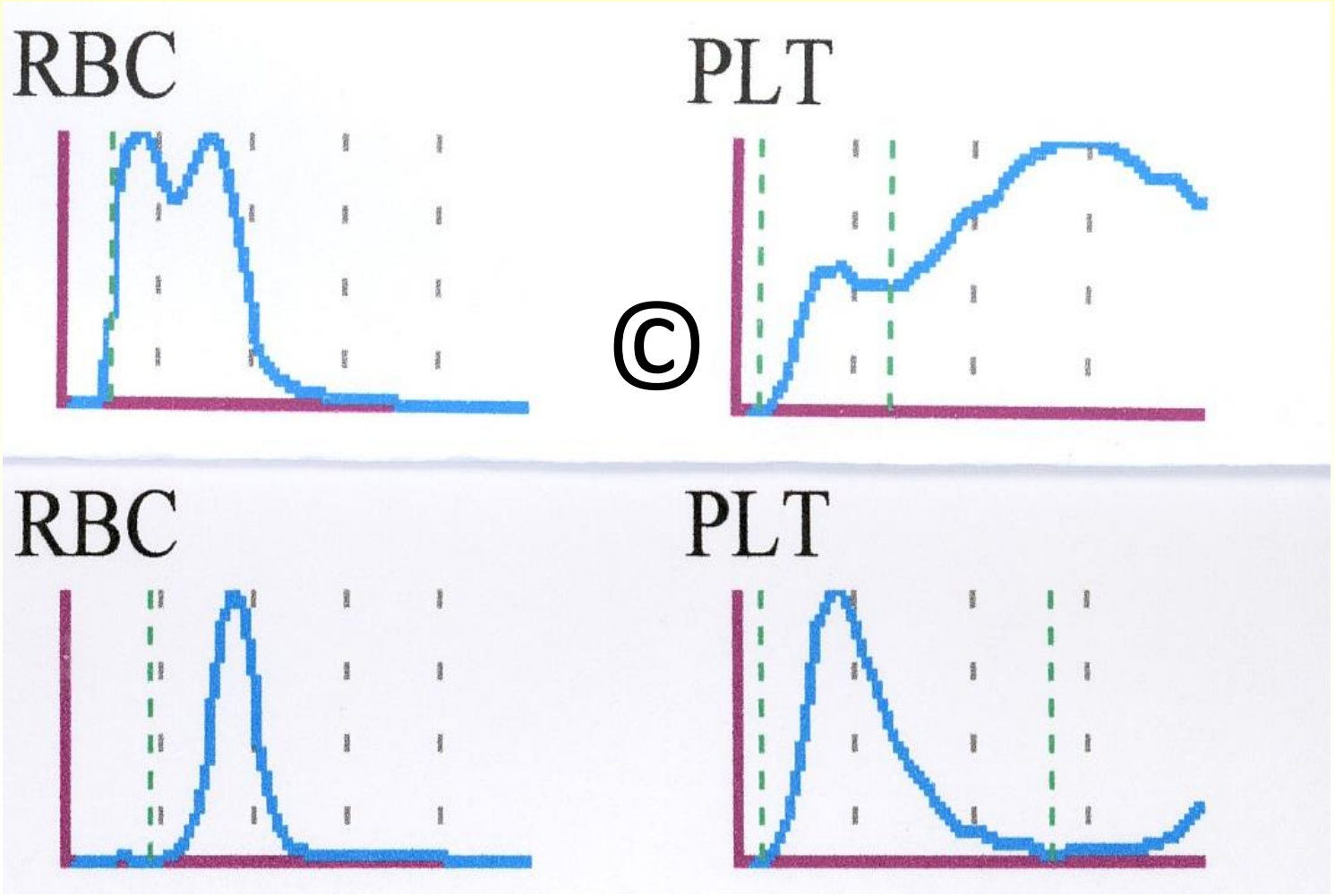
… not always so simple

- Clinical history – baggage handler at Brussels International airport
- Blood film



Tournoy et al. (2006) Haemolysis, elevated liver enzymes, and thrombocytopenia in a 34-year-old pregnant woman. *Lancet*, **368**, 90.

Fragmentation of normal cells is not always MAHA



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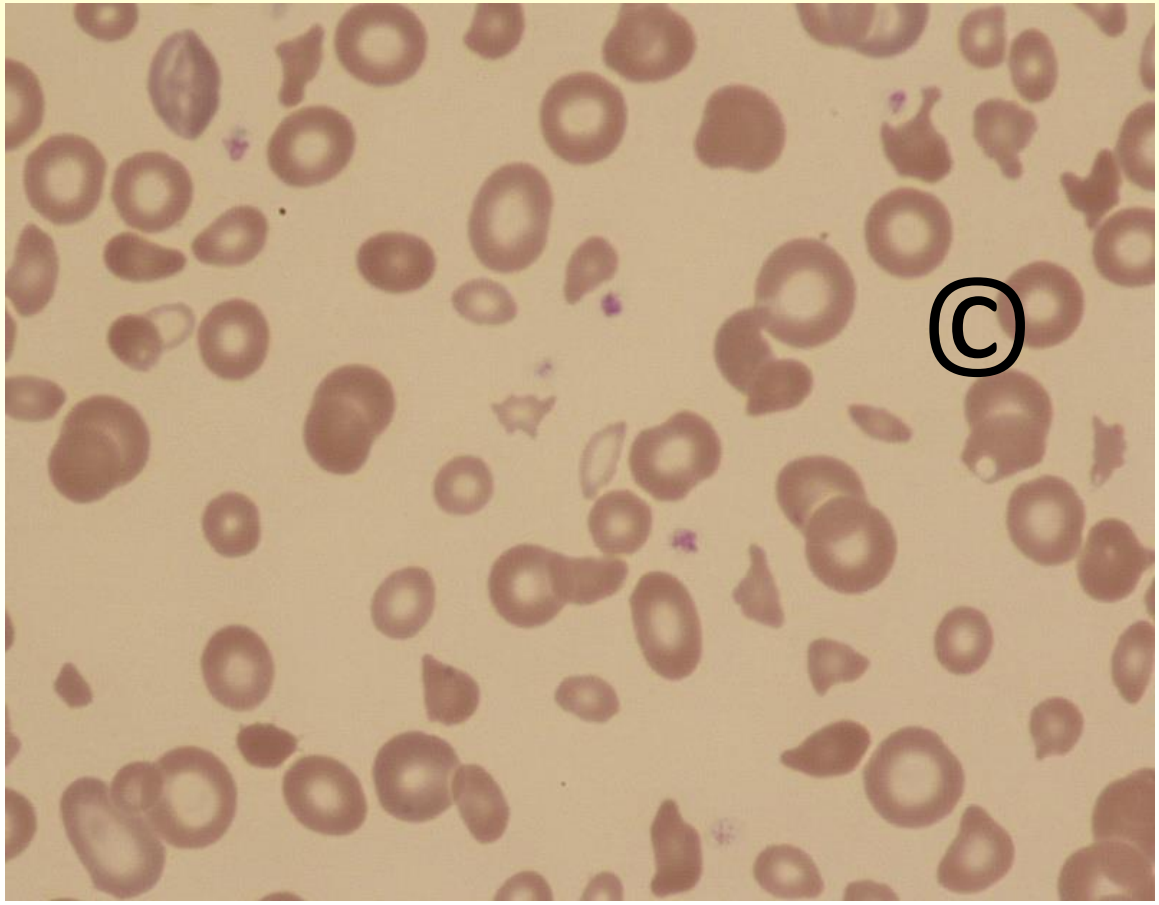
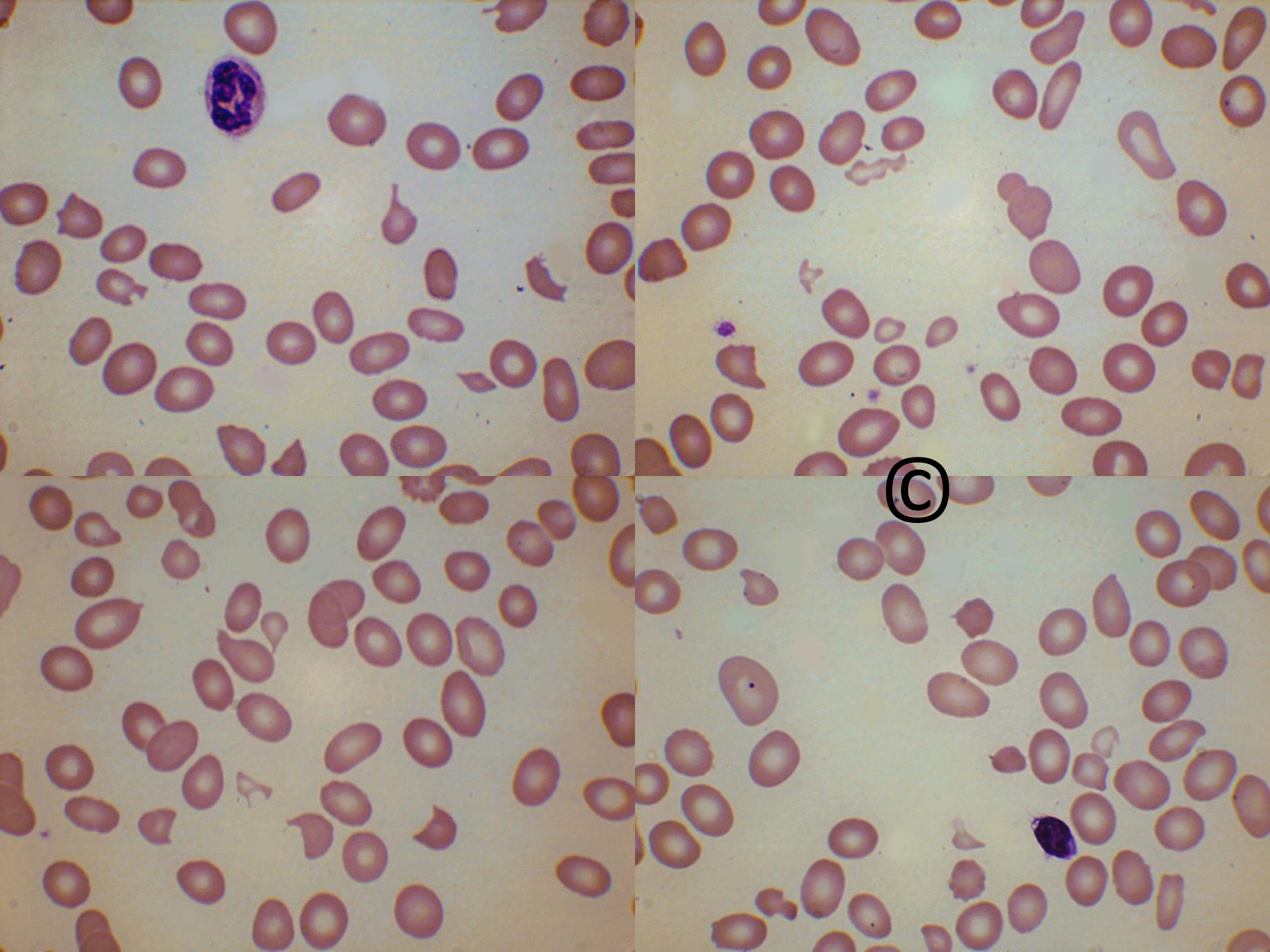


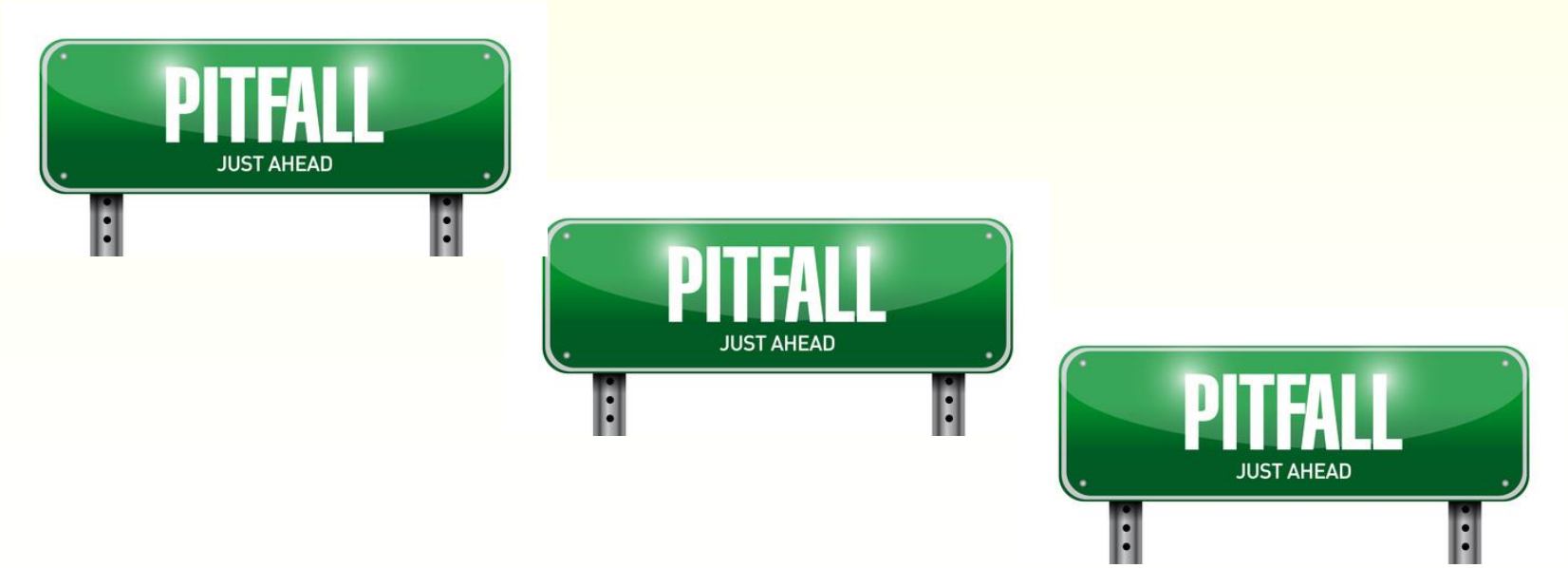
Bain BJ, Varu V, Rowley M and Foale R (2015) *Am J Hematol*, **90**, 1179.

Beware – megaloblastic anaemia has fragments



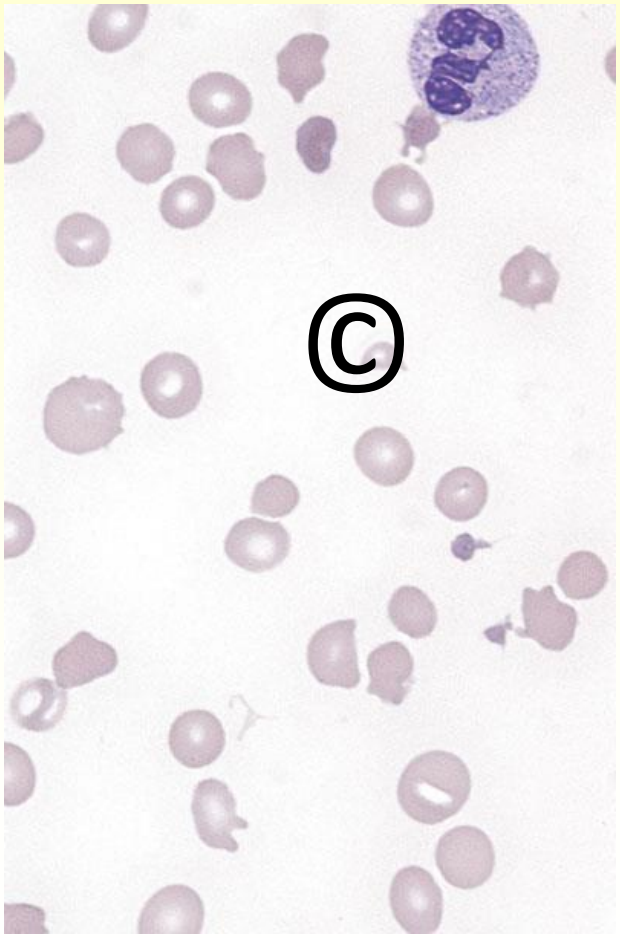
Beware – abnormal cells may fragment

- Could fragments be the result of dyserythropoiesis, a defective red cell membrane or a haemoglobinopathy?



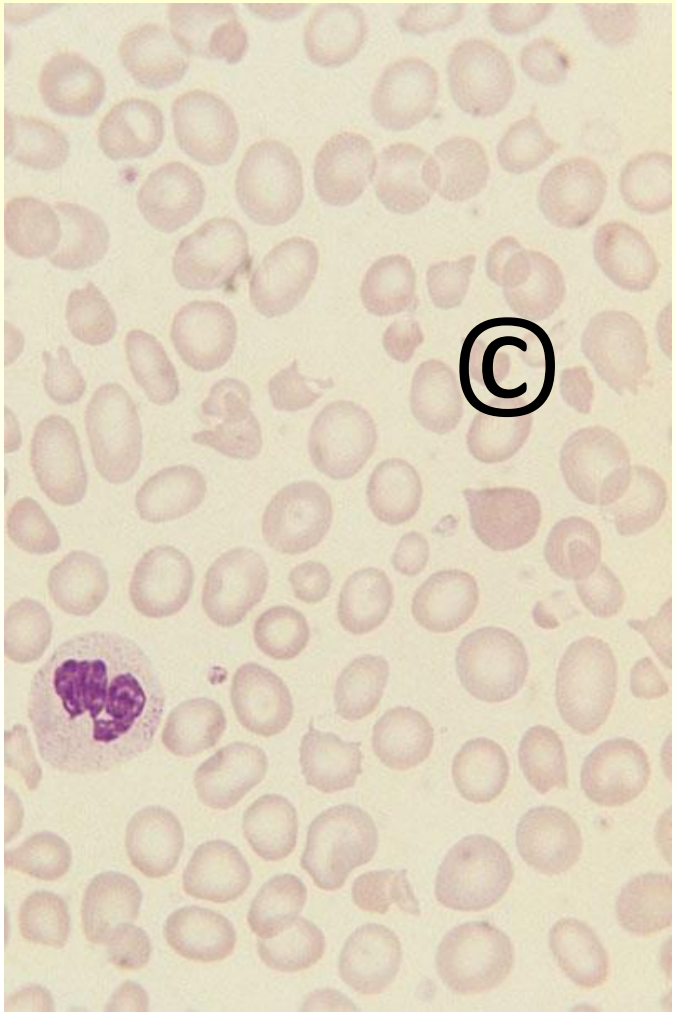
Beware – abnormal cells may fragment

- This anaemic, thrombocytopenic patient was treated by plasma exchange for ‘TTP’
- The diagnosis was actually MDS



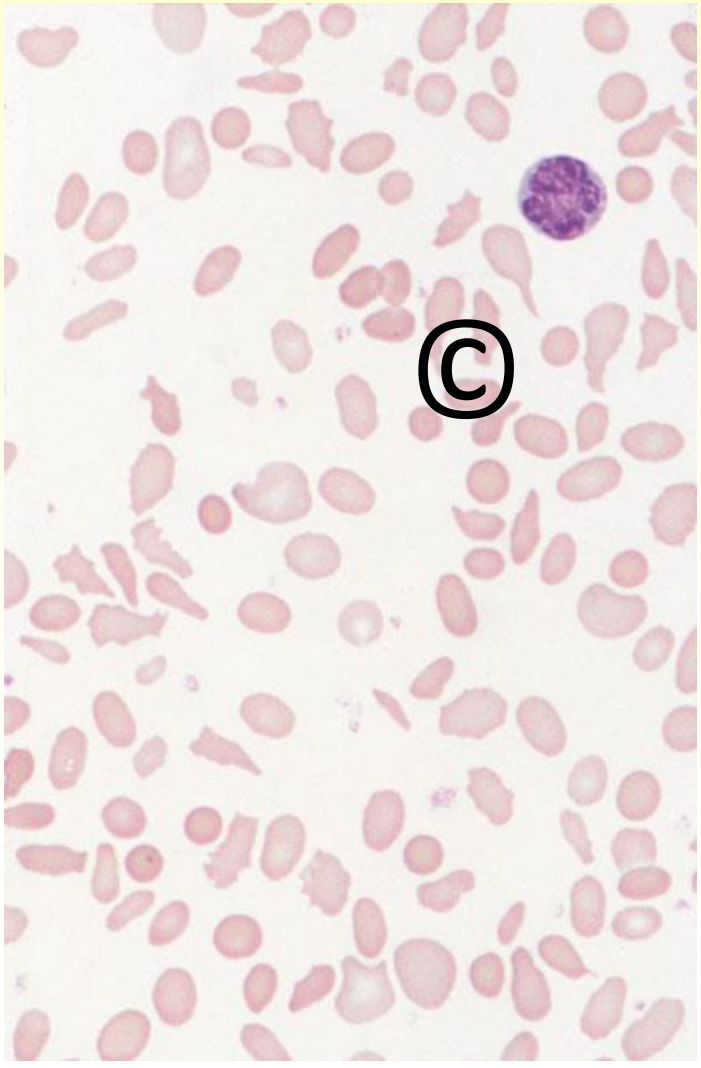
Beware – abnormal cells may fragment

- Haemoglobin H disease

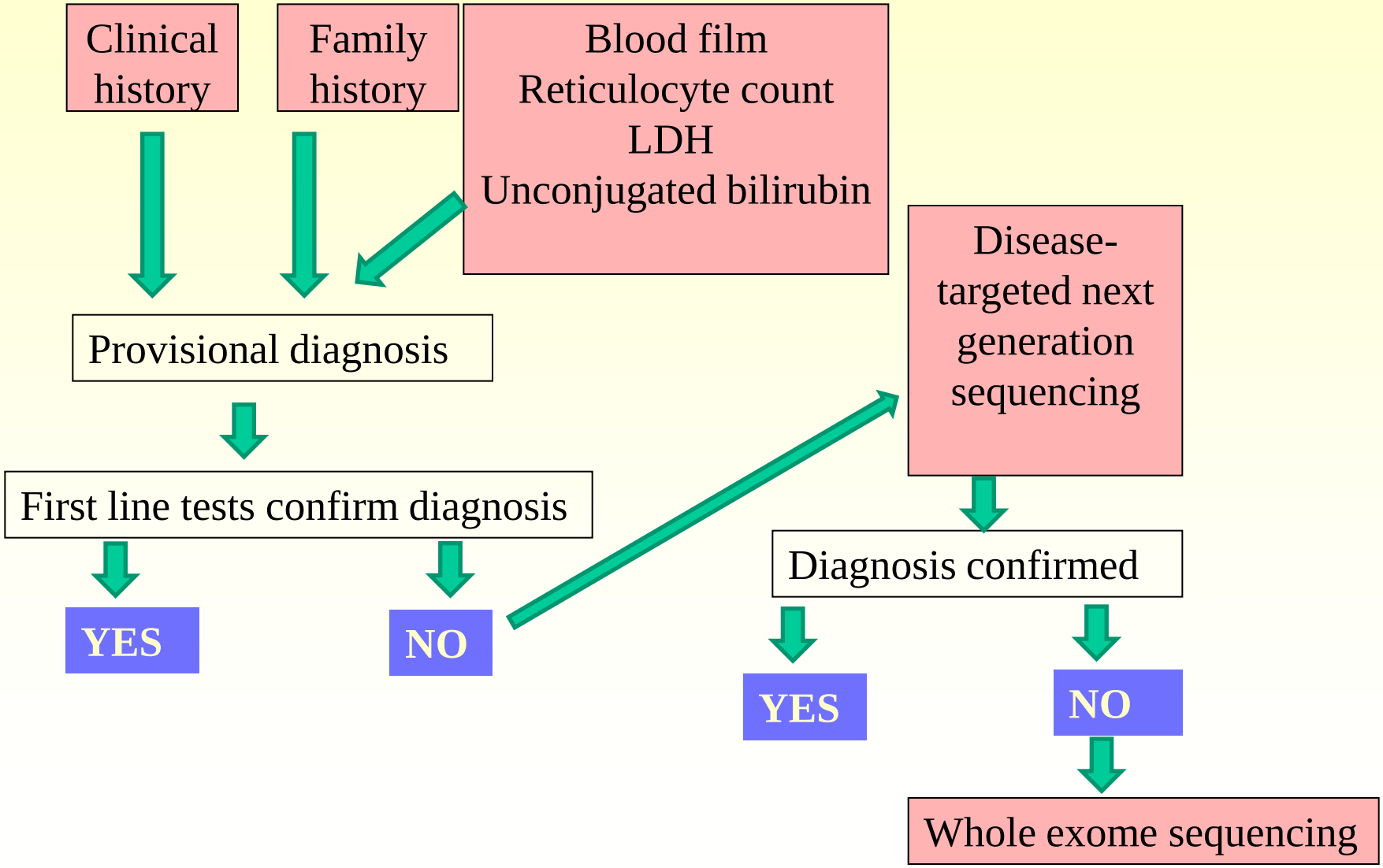


Beware – abnormal cells may fragment

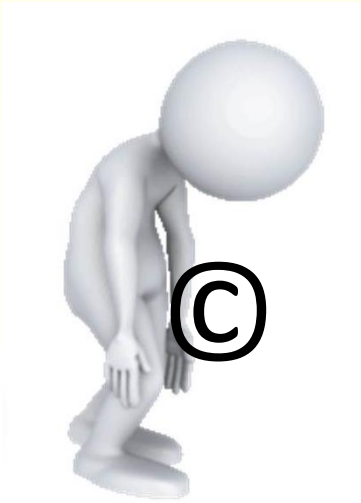
- Hereditary pyropoikilocytosis



Flow chart for suspected inherited haemolytic anaemia

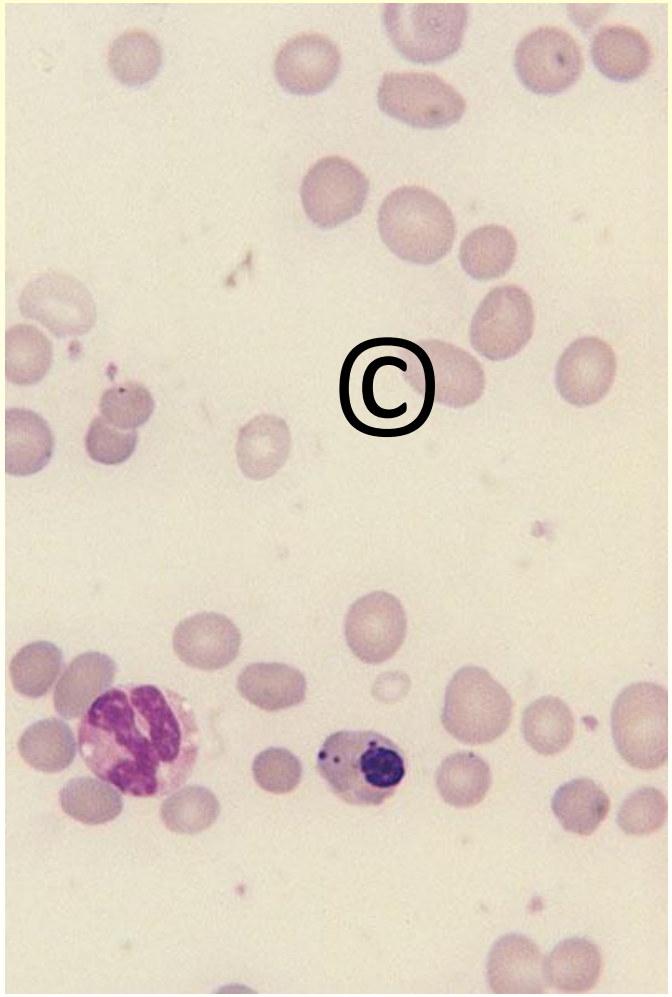


You know the patient has a red cell disorder but things have got worse



Why has the anaemia got worse?

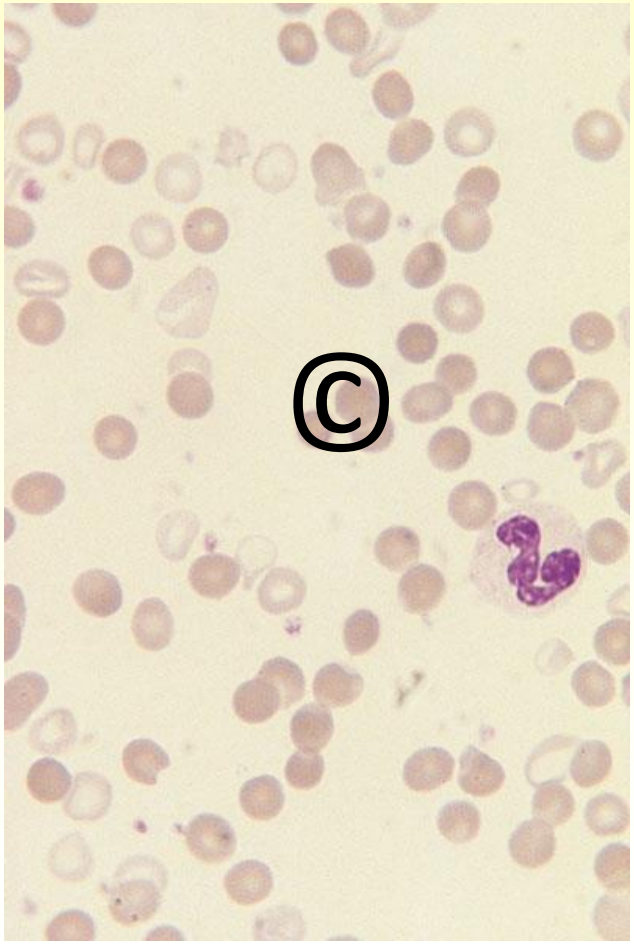
- An elderly woman with known hereditary spherocytosis
- Why is she suddenly more anaemic?



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

Why has the anaemia got worse?

- She is anaemic again
- What has happened this time?



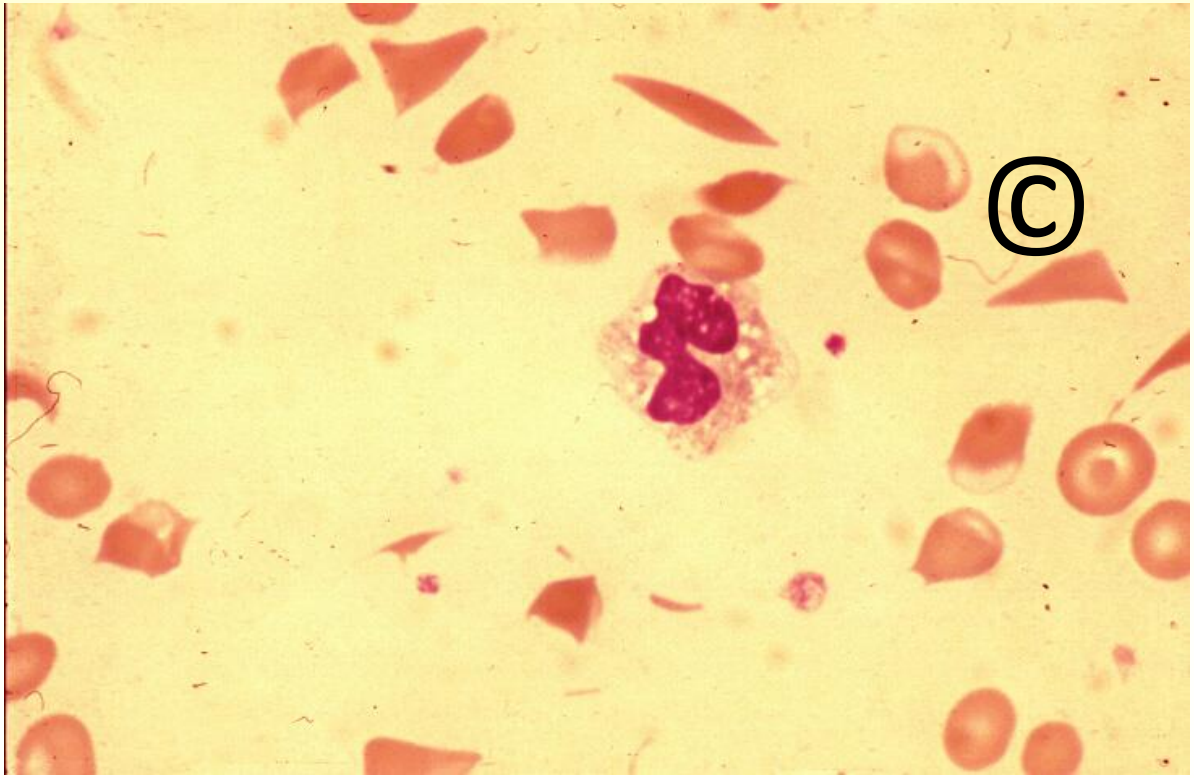
Bain BJ, 2014, Interactive Imagebank, 2nd Edn, Wiley-Blackwell

You know the patient has sickle cell disease – is something else going on?

- Acute worsening of anaemia
  - Splenic sequestration
  - Parvovirus B19
  - Sickle crisis
  - Hyperhaemolysis following transfusion
  - Something else

You know the patient has sickle cell disease – is something else going on?

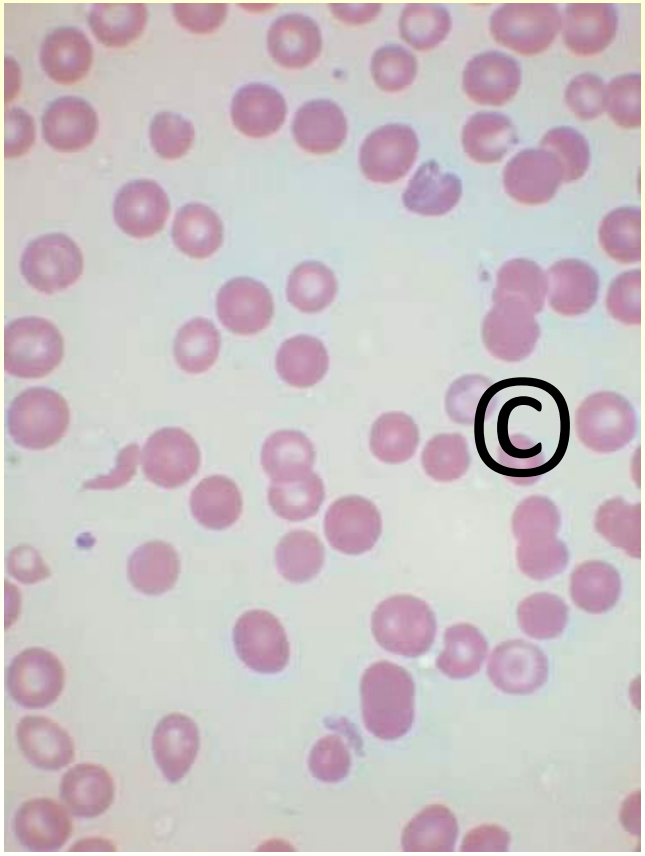
- Acute sickle crisis with hypoxia

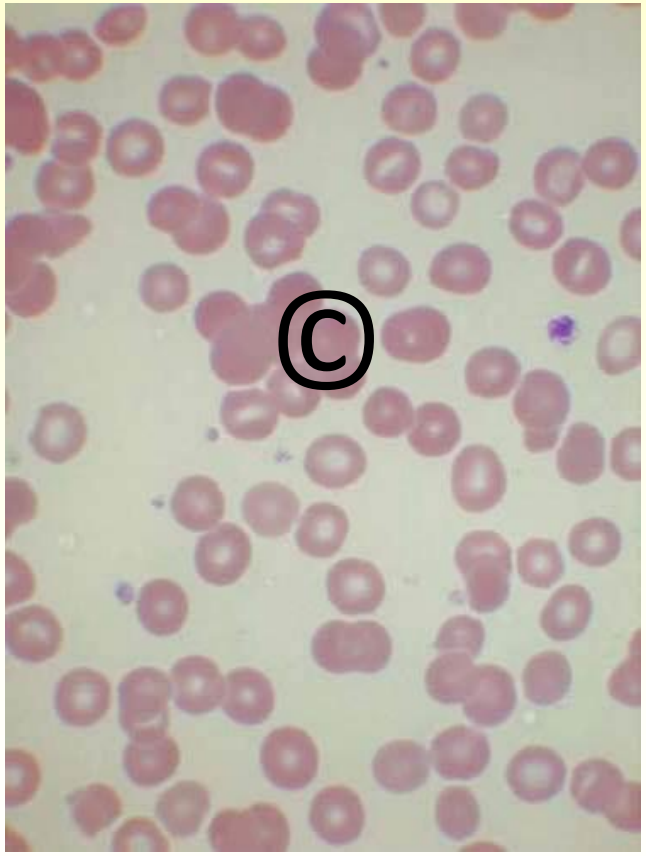


You know the patient has sickle cell disease – is something else going on?

- This 82-year-old Afro-Caribbean woman with unusually mild sickle cell/haemoglobin C disease was admitted with pneumonia and myocardial infarction
- Hb fell from 95 to 74 g/l
- What is going on?

You know the patient has sickle cell disease – is something else going on?



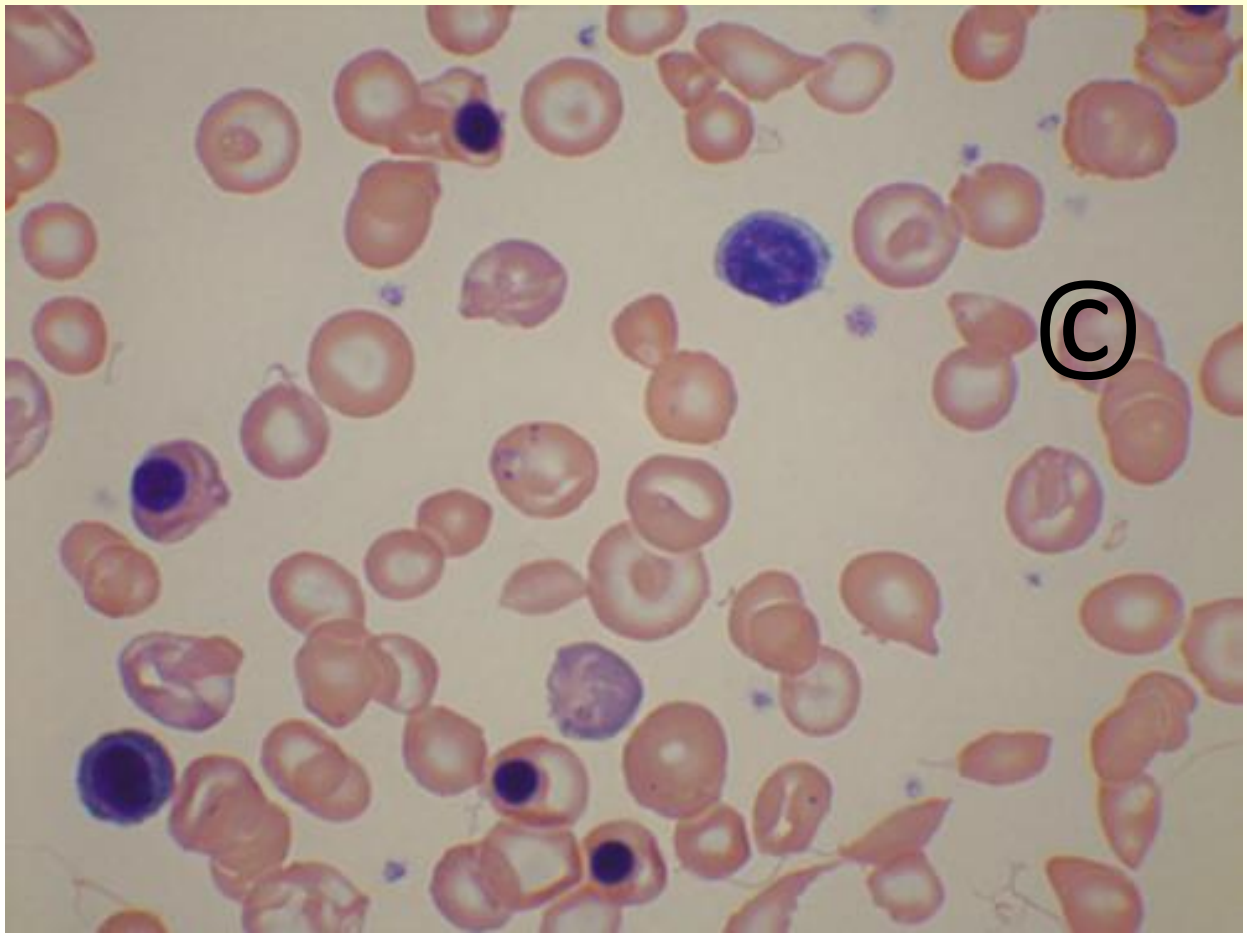


- What is going on?

You know the patient has sickle cell disease – is something else going on?

- Routine antenatal film from a 37-year-old Nigerian woman
- She admits to having sickle trait
- FBC:
  - 'Leucocytes' 112 × 10^9/l
  - Hb 55 g/l
  - MCV 101 fl
  - Platelets 471 × 10^9/l

You know the patient has sickle cell disease – is something else going on?



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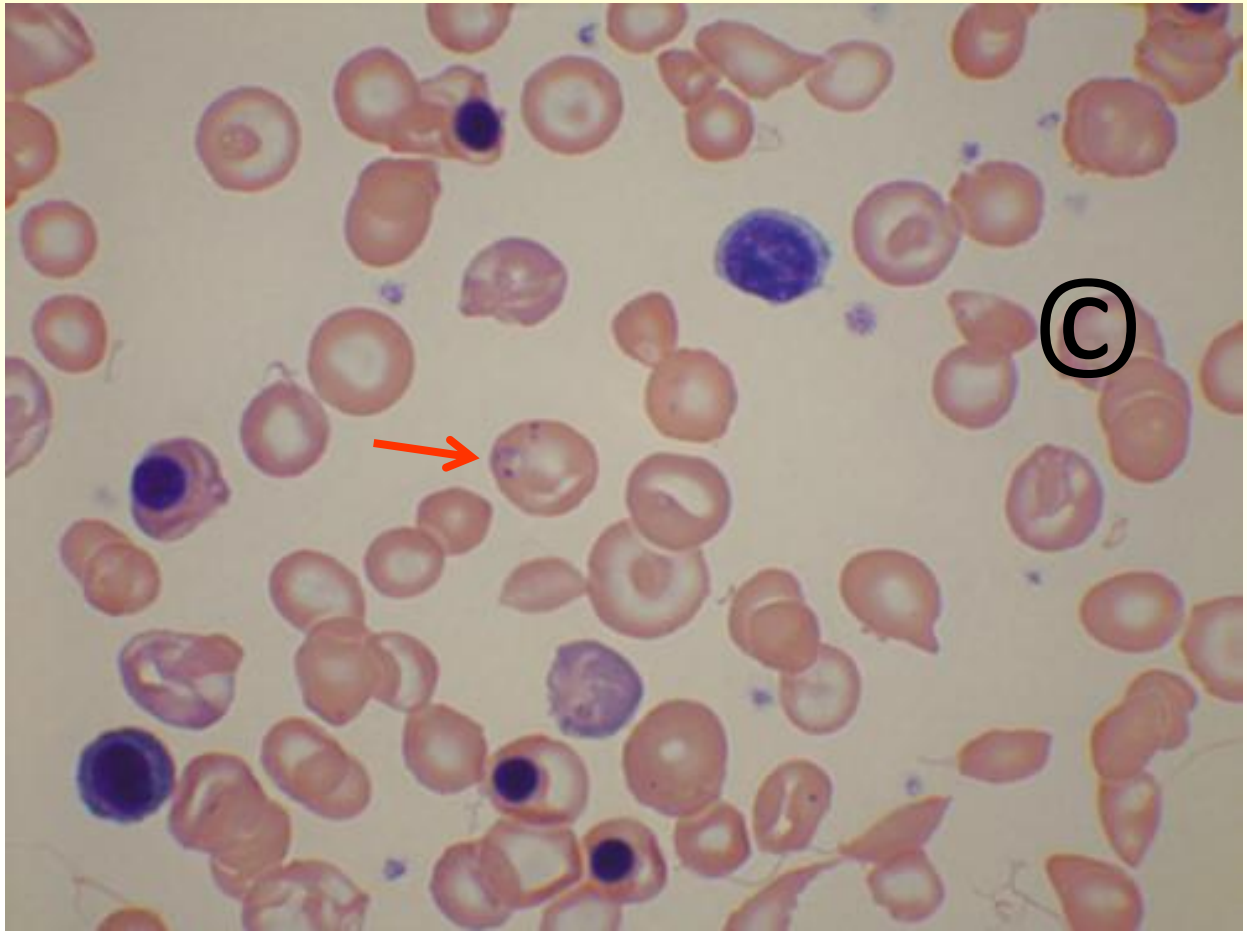

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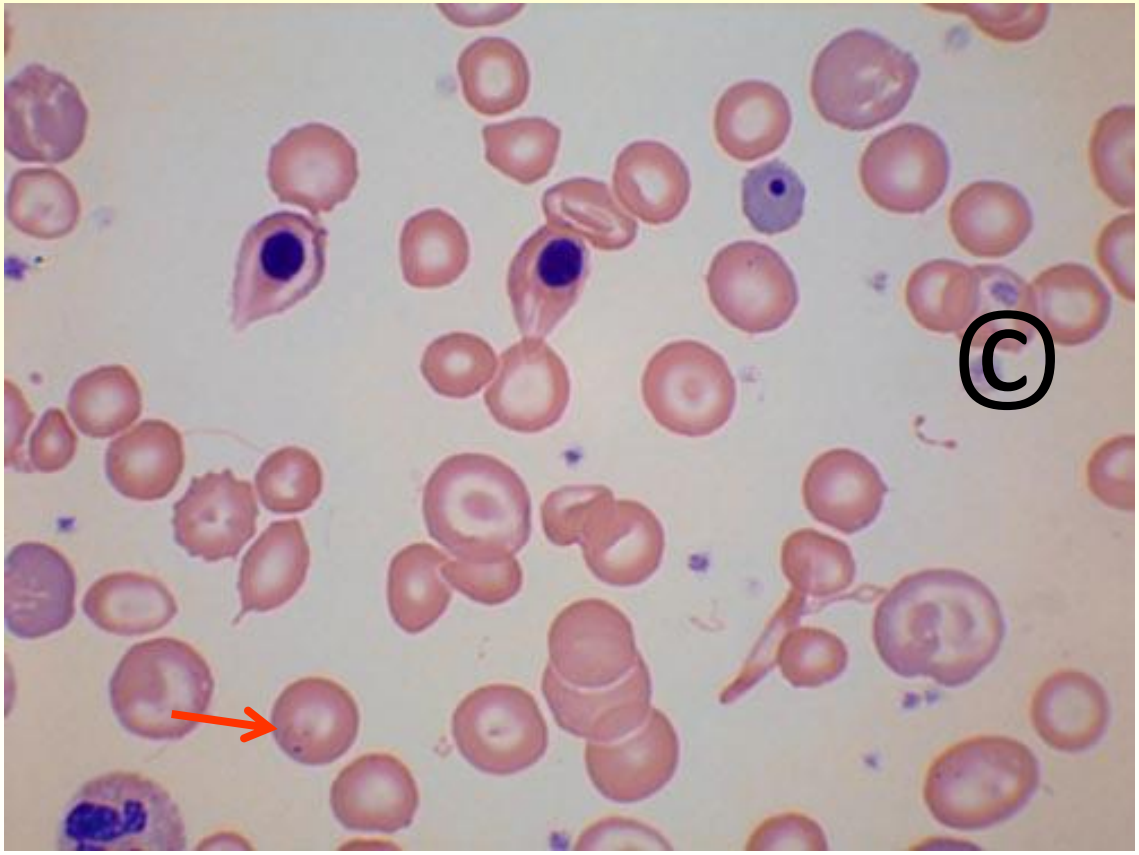

Conclusions

- Red cell diagnosis is clinically important and can be intellectually rewarding
- A blood film remains important but should be integrated with modern diagnostic methods
- Pitfalls may be avoided by diligence and thoughtfulness

The End