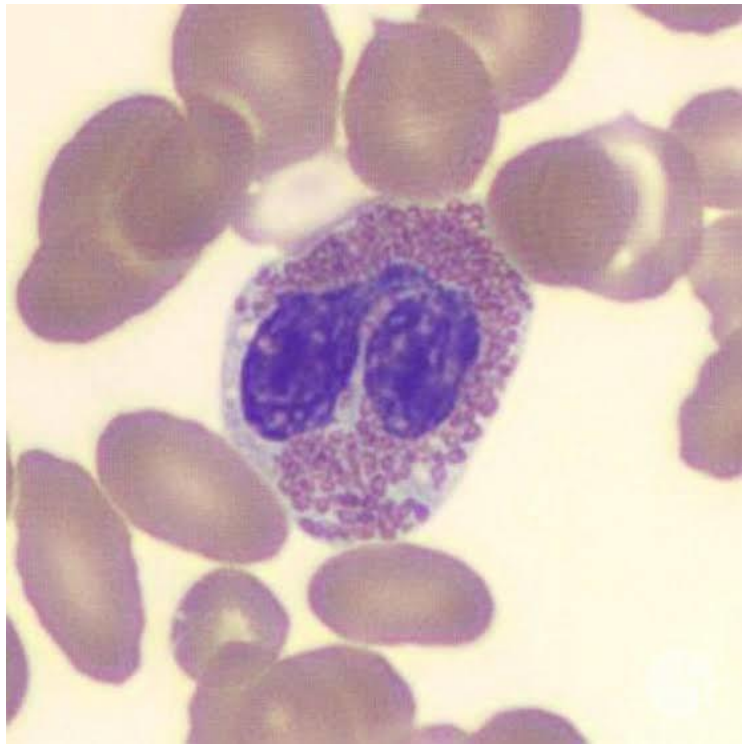
My favourite blood cell

Peter Carey

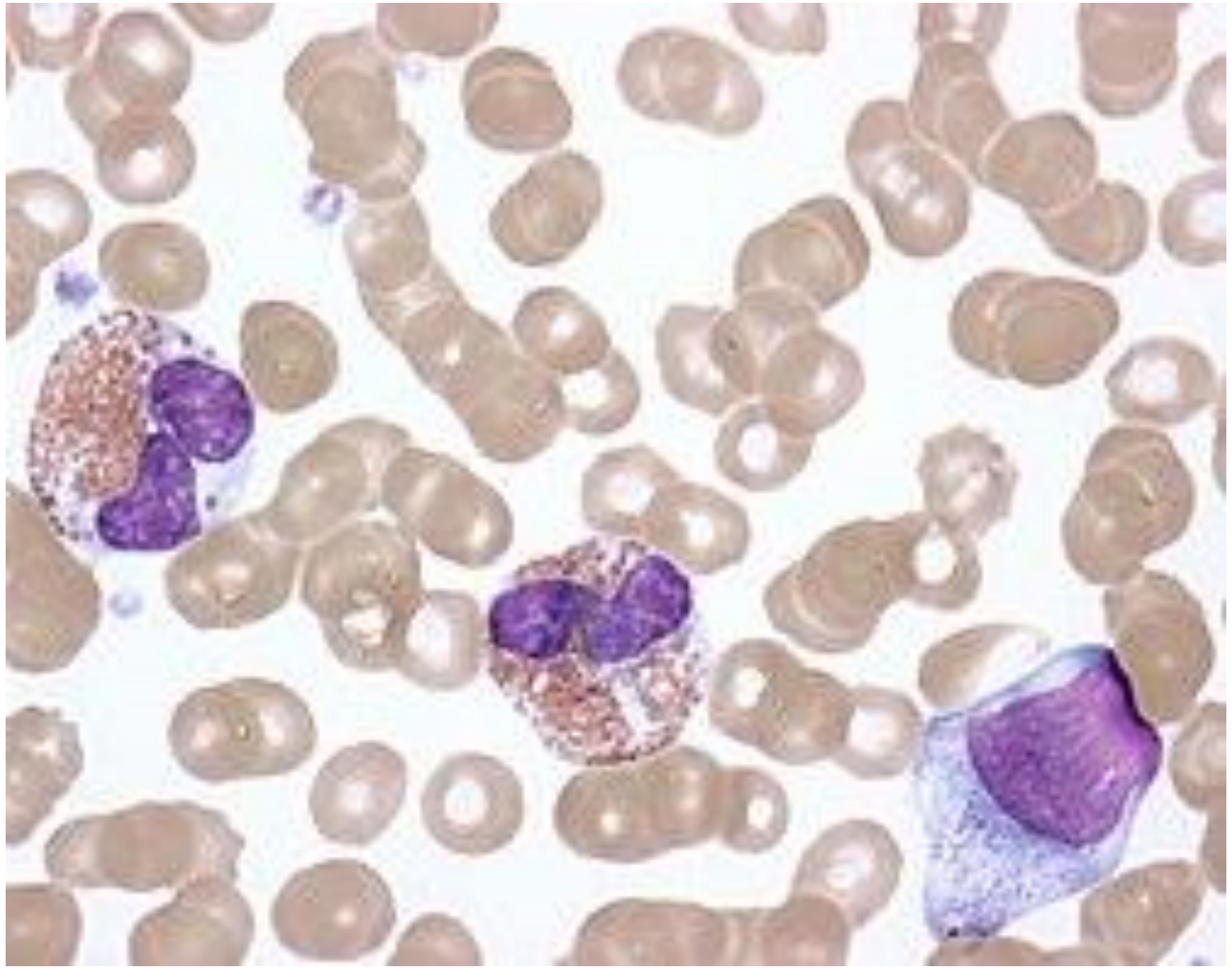
Royal Victoria Infirmary, Newcastle upon Tyne, UK

The humble Eosinophil



Why?

- Uniquely colourful, recognizable, pretty cell
- Enigmatic function in health and disease
- A personal issue with eosinophils
- A most memorable adult patient
- ‘Sentinel’ role for microscopists



Functions

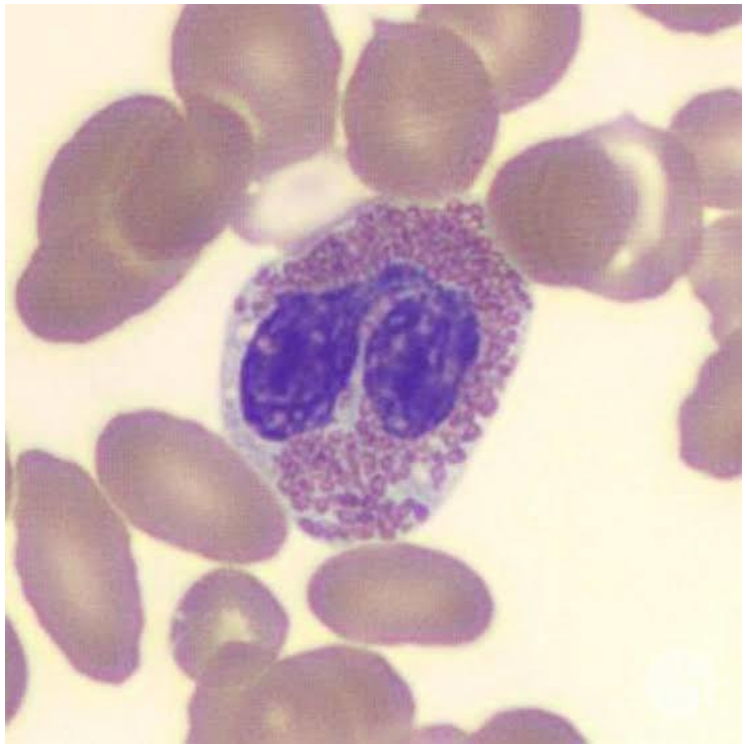
Immune response particularly to parasitic and viral infections through the production of granule proteins, lipid mediators, enzymes, growth factors and cytokines

Regulation of mechanisms associated with allergy

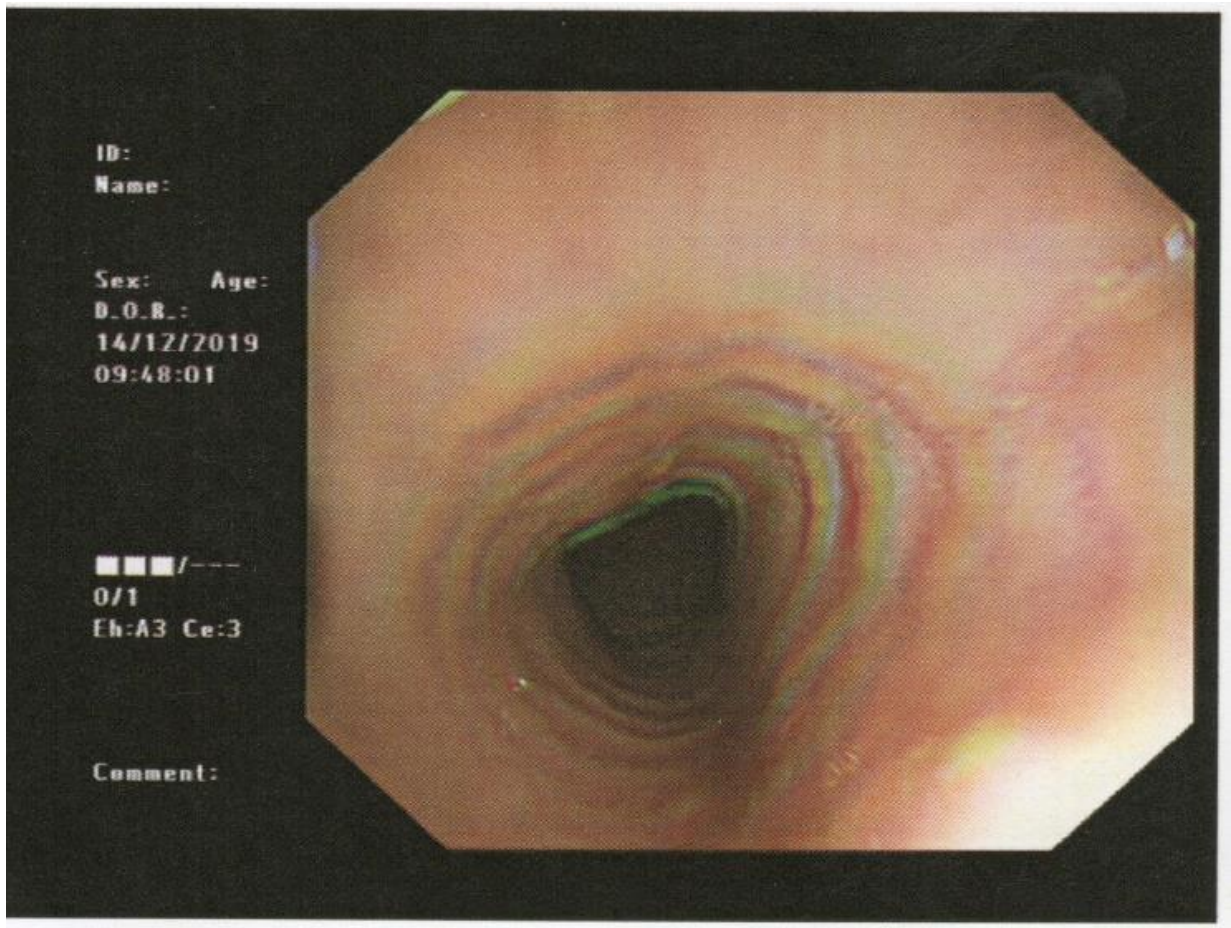
Fibrin removal during inflammation

Antigen presentation to T cells

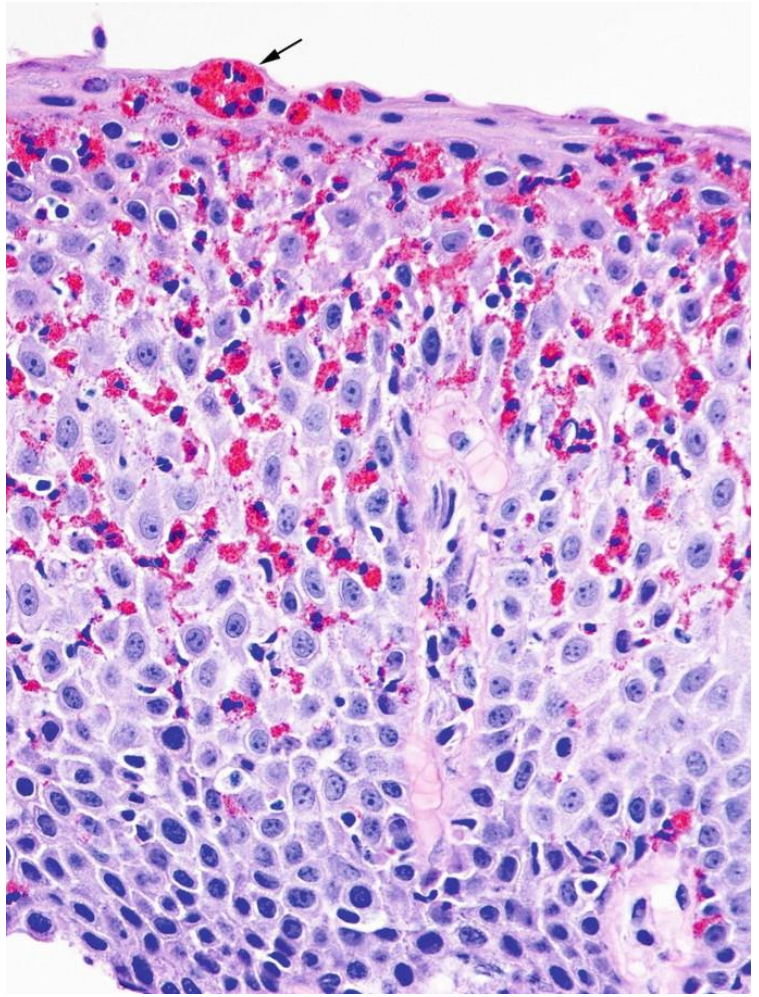
Present in many tissues as well as peripheral blood, marrow and lymph glands



Any ideas?



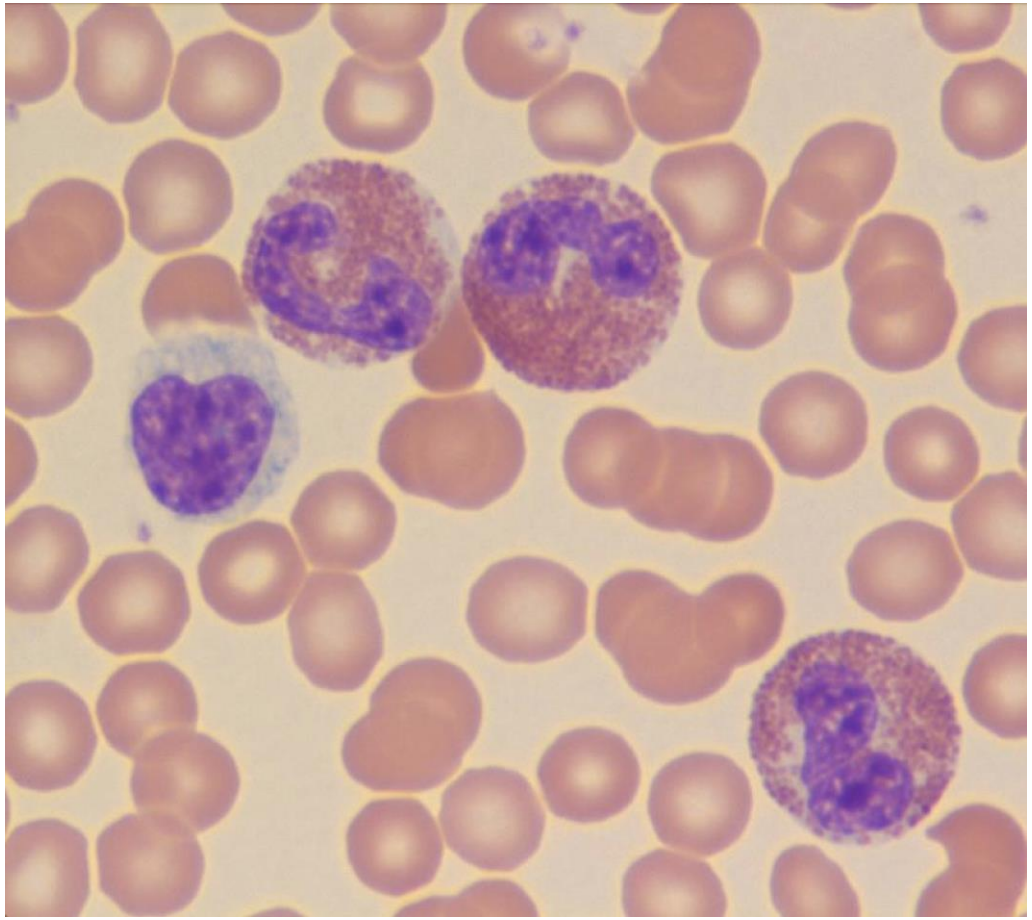
Eosinophilic oesophagitis

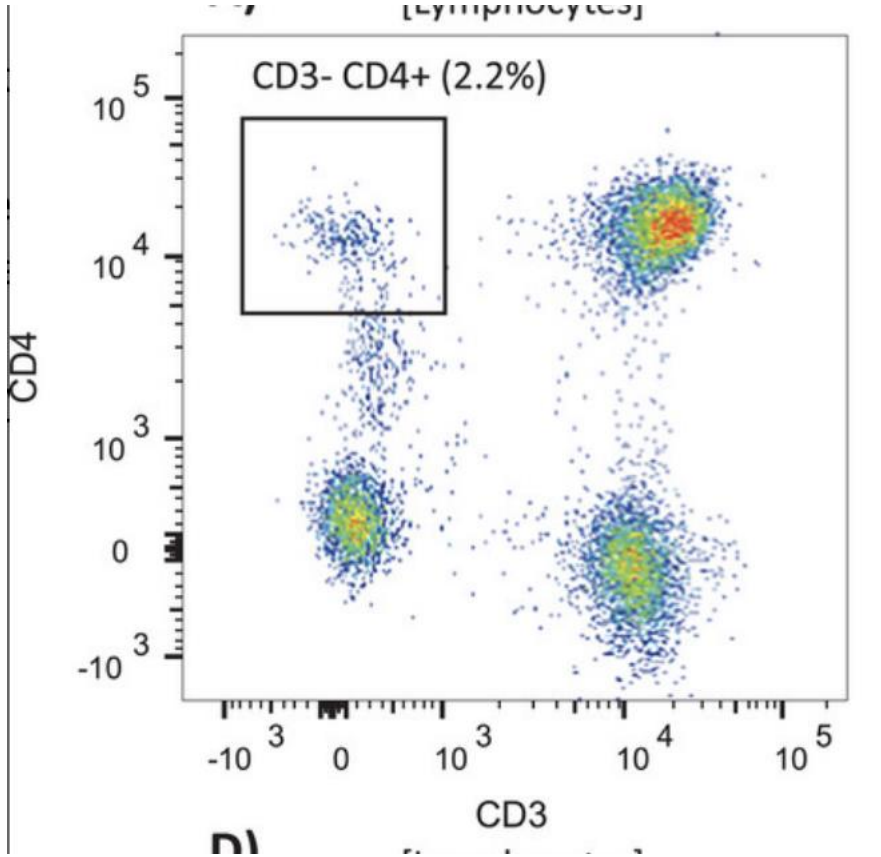


A memorable patient

- 1990’s Sunderland
  - Age 24, male, computer engineer
    - Minor fluctuating eosinophilia
    - Fluctuating itchy maculopapular skin rash
    - No history of allergy, atopy or asthma; normal CXR
    - No evidence of parasitic infestation
    - No evidence of myeloproliferative disorder
    - No adenopathy or organomegaly; no evidence of Hodgkin Lymphoma
    - Normal marrow karyotype

CD4+ T cell clone with aberrant loss of CD3 Lymphocytic Hypereosinophilic syndrome



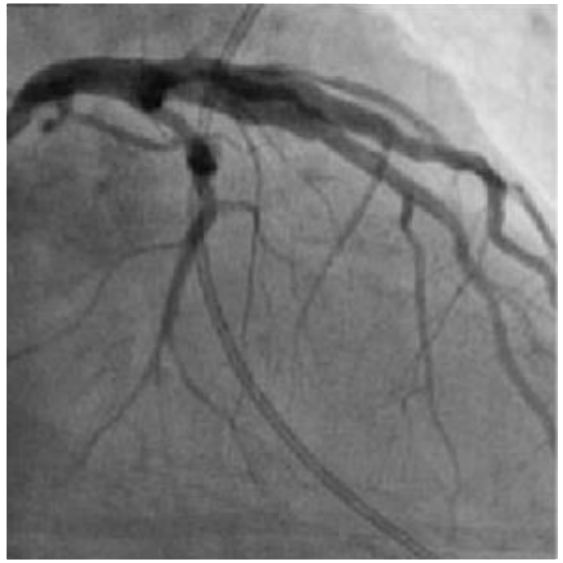


Follow up

- Fluctuating flares – responsive to sparing topical or oral steroid
- Cambridge – early 2000's - observation continues
  - Plan to use Alemtuzumab (Campath)
- Newcastle 2008
  - Subcutaneous Alemtuzumab – good initial response, then development of neutralizing antibodies
  - Mepolizumab (IL-5 inhibitor) – clinical trial followed by compassionate use

Complications

- 2012 Angina
- Eosinophilic aneurysms coronary arteries

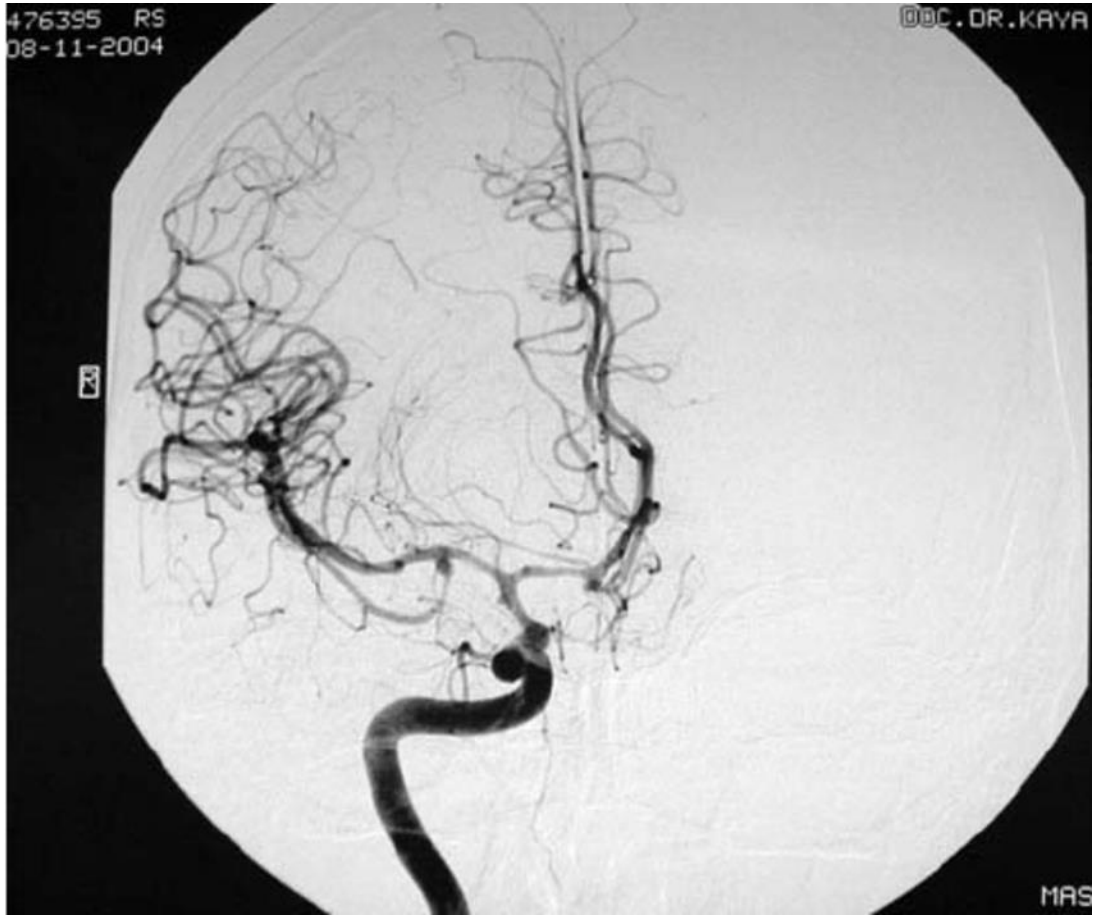


- Consideration of CABG
  - ?BMT allograft first

Complications (2)

- Acute diplopia
  - IVth cranial nerve palsy
- Cerebral aneurysms

- BM allograft
  - Fatal cardiac event during conditioning



Who is this?

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Honey Guide (Indicator indicator)

Eosinophils as indicators (for microscopists)

- Reminder to check film at low power
  - Especially NEQAS or exam apparently normal film:

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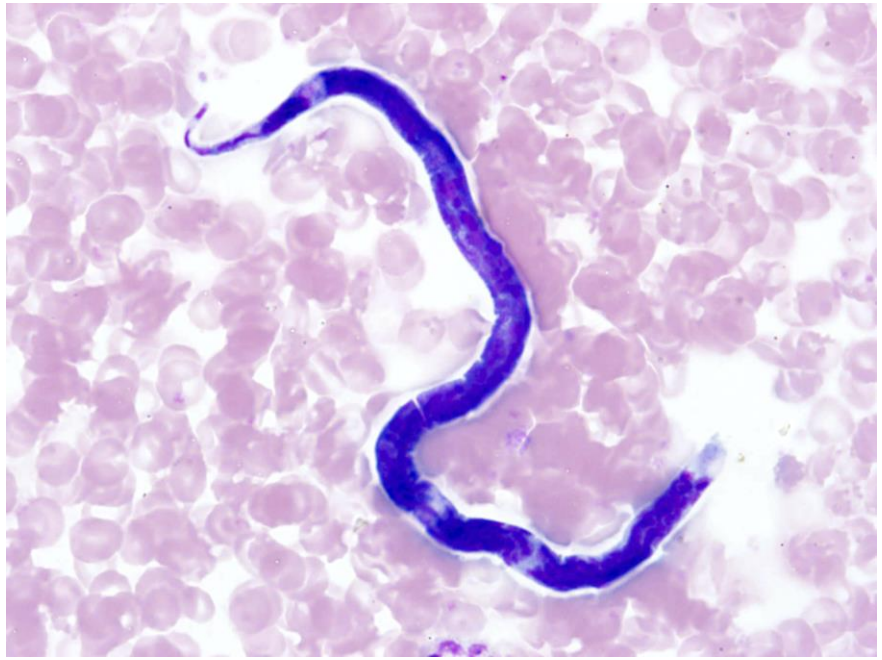
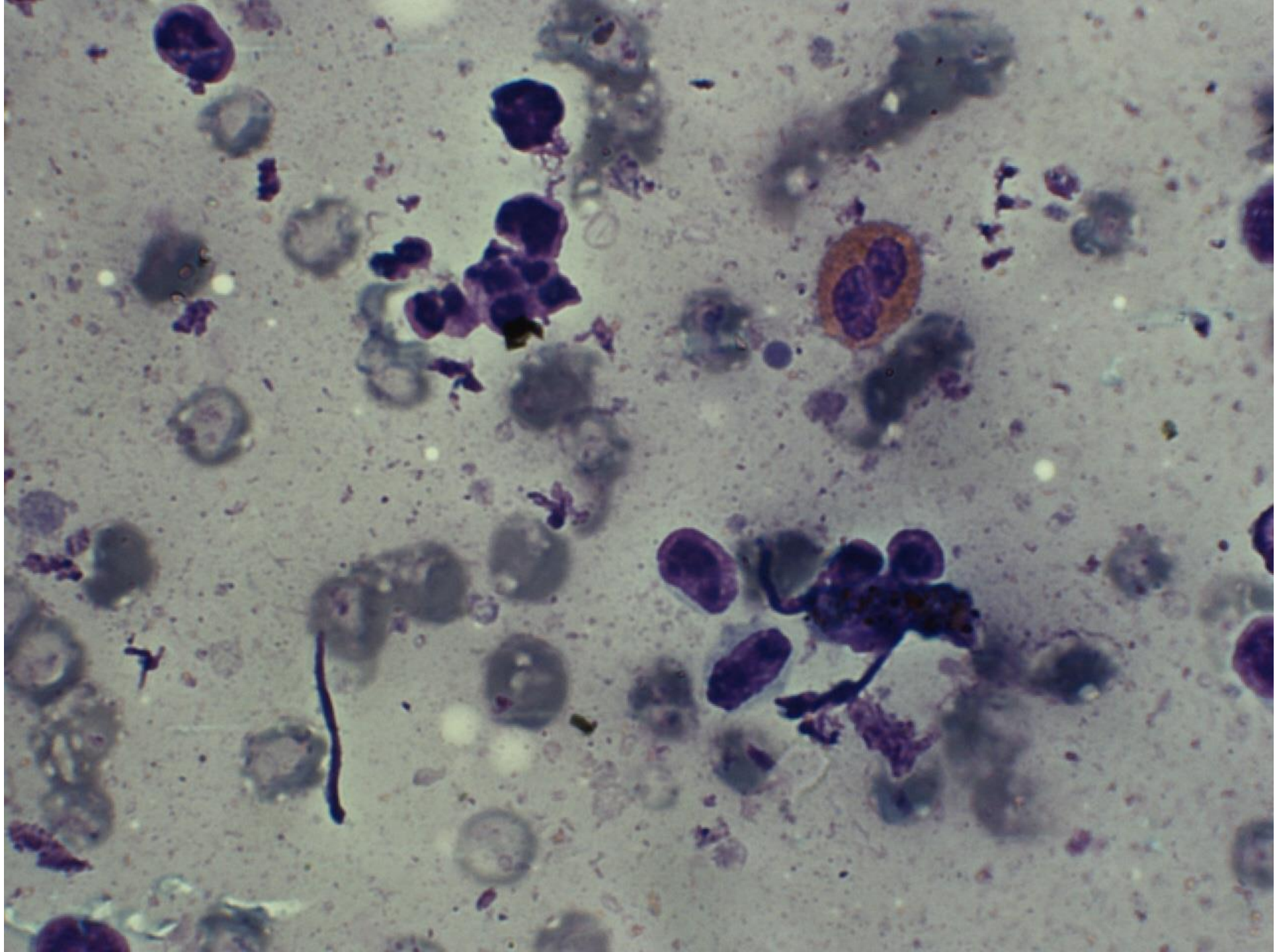
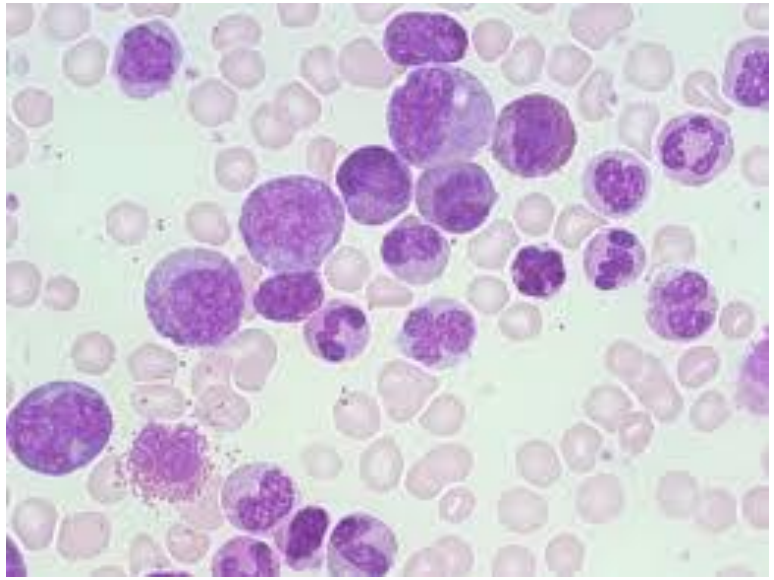


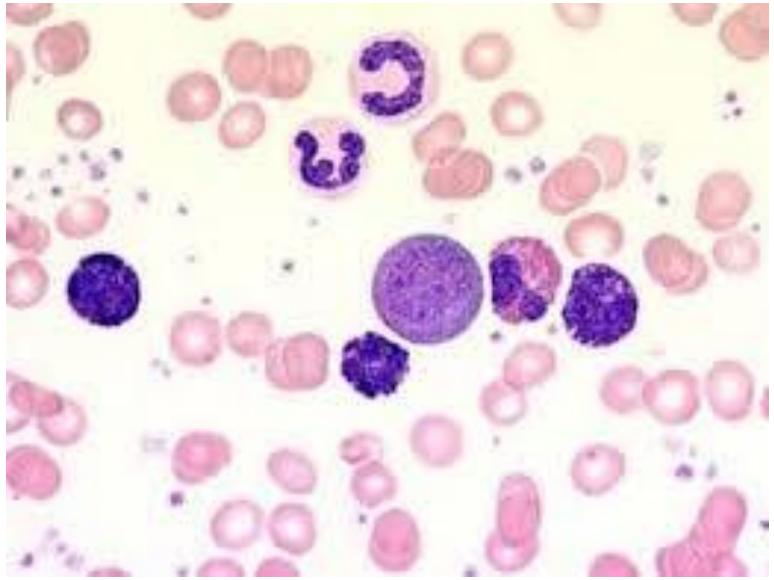


Eosinophils as indicators (for microscopists)

- In PB
  - Parasites
  - Myeloproliferative neoplasms
    - *BCR::ABL*
    - *PDGFRA, PDGFRB, FGFR1, JAK2,FLT3,ETV6::ABL1*
  - Hodgkin Lymphoma
- In BM aspirates
  - Leukaemias
    - AML with inv16; t(8;21) (Eosinophils with some basophilic granules)
- In BM trephines
  - With fibrosis
    - Systemic Mastocytosis
    - Hodgkin Lymphoma

CML with *BCR::ABL1*





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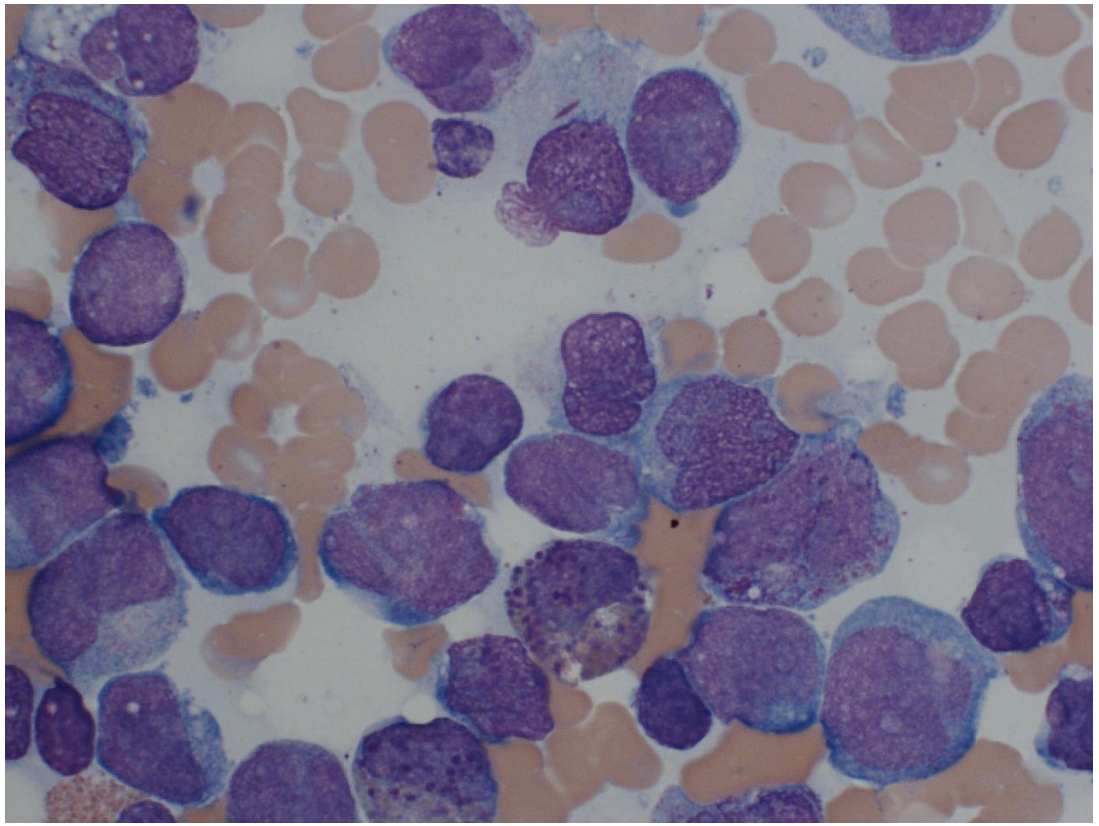
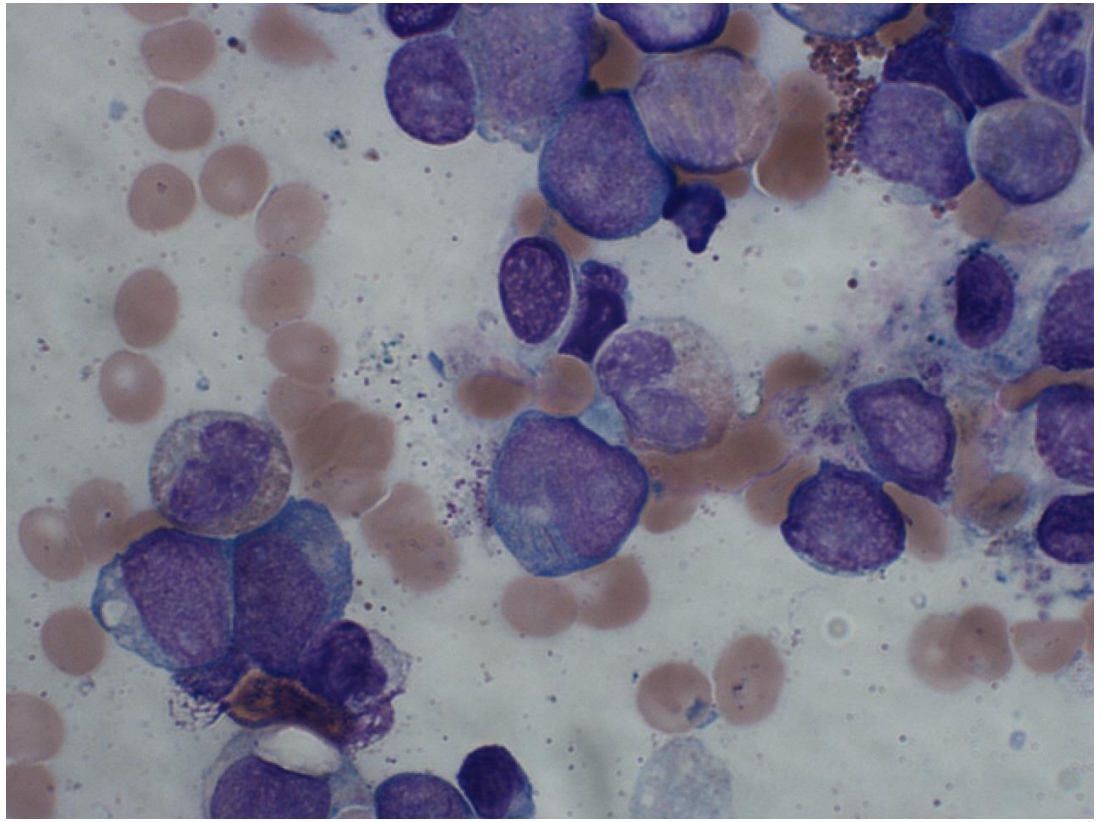
AML with *CBFB::MYH11*
inv(16) [AMML M4Eo]



AML with *RUNX1::RUNX1T1*
t(8;21)



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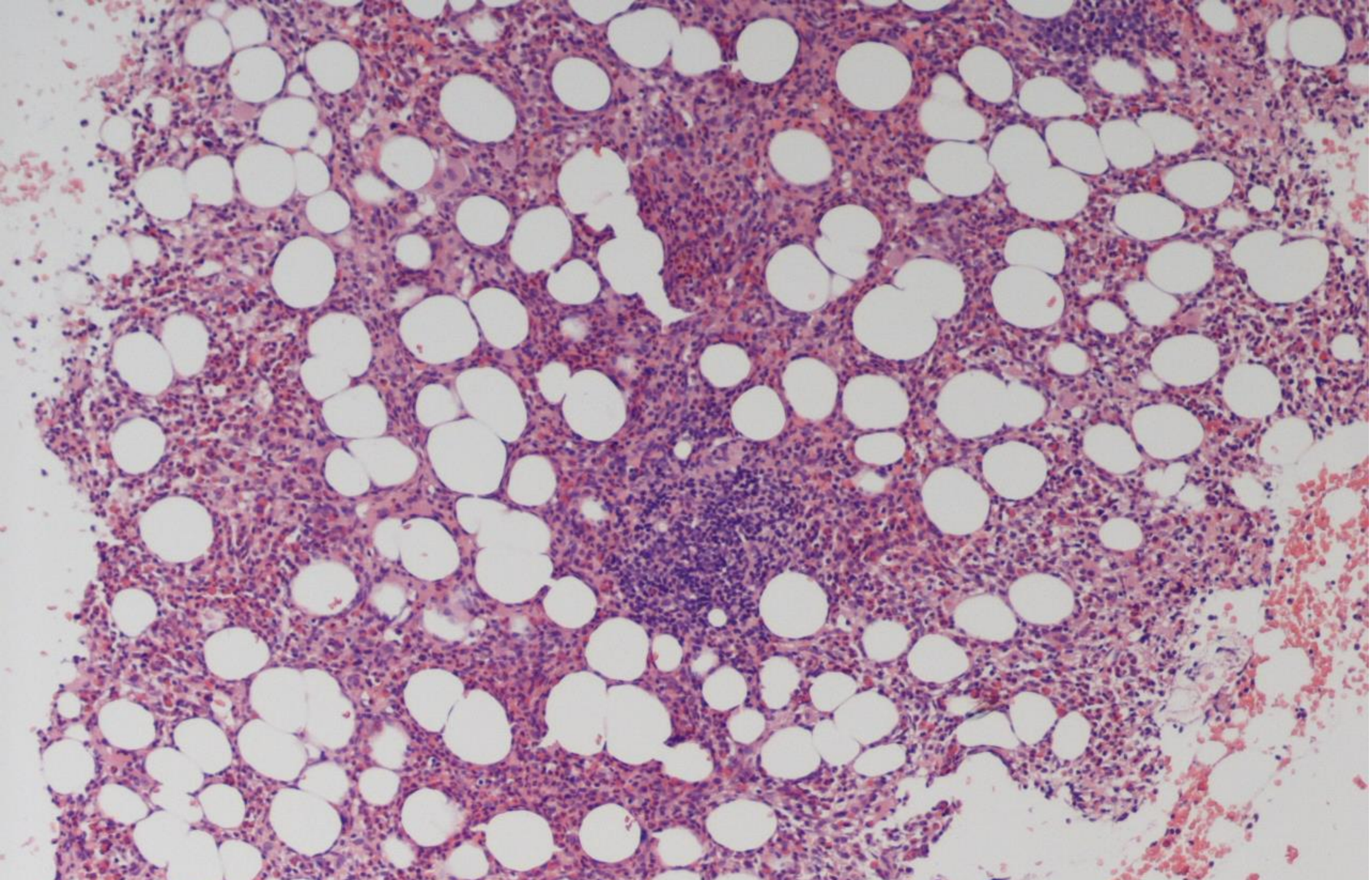

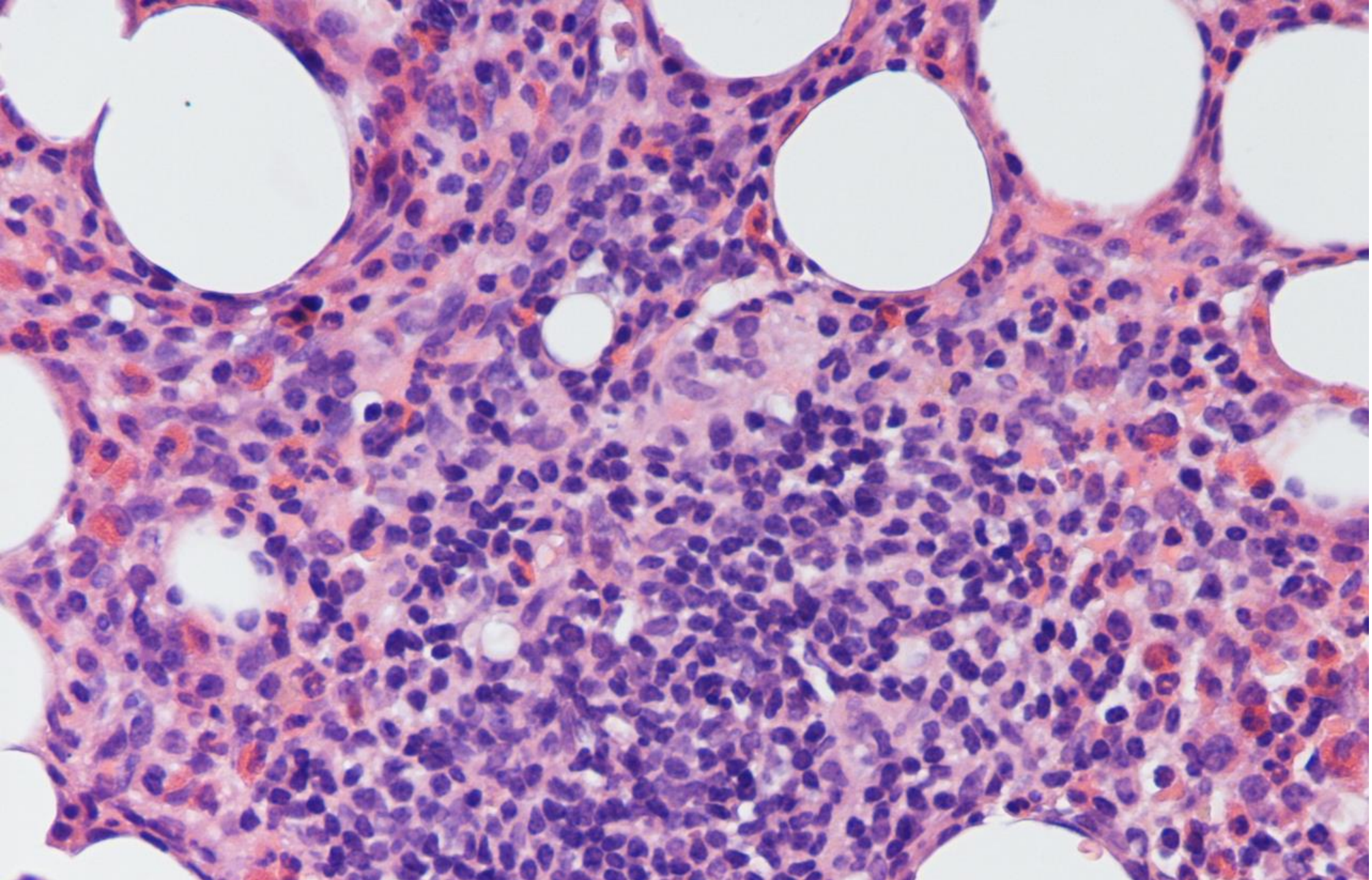

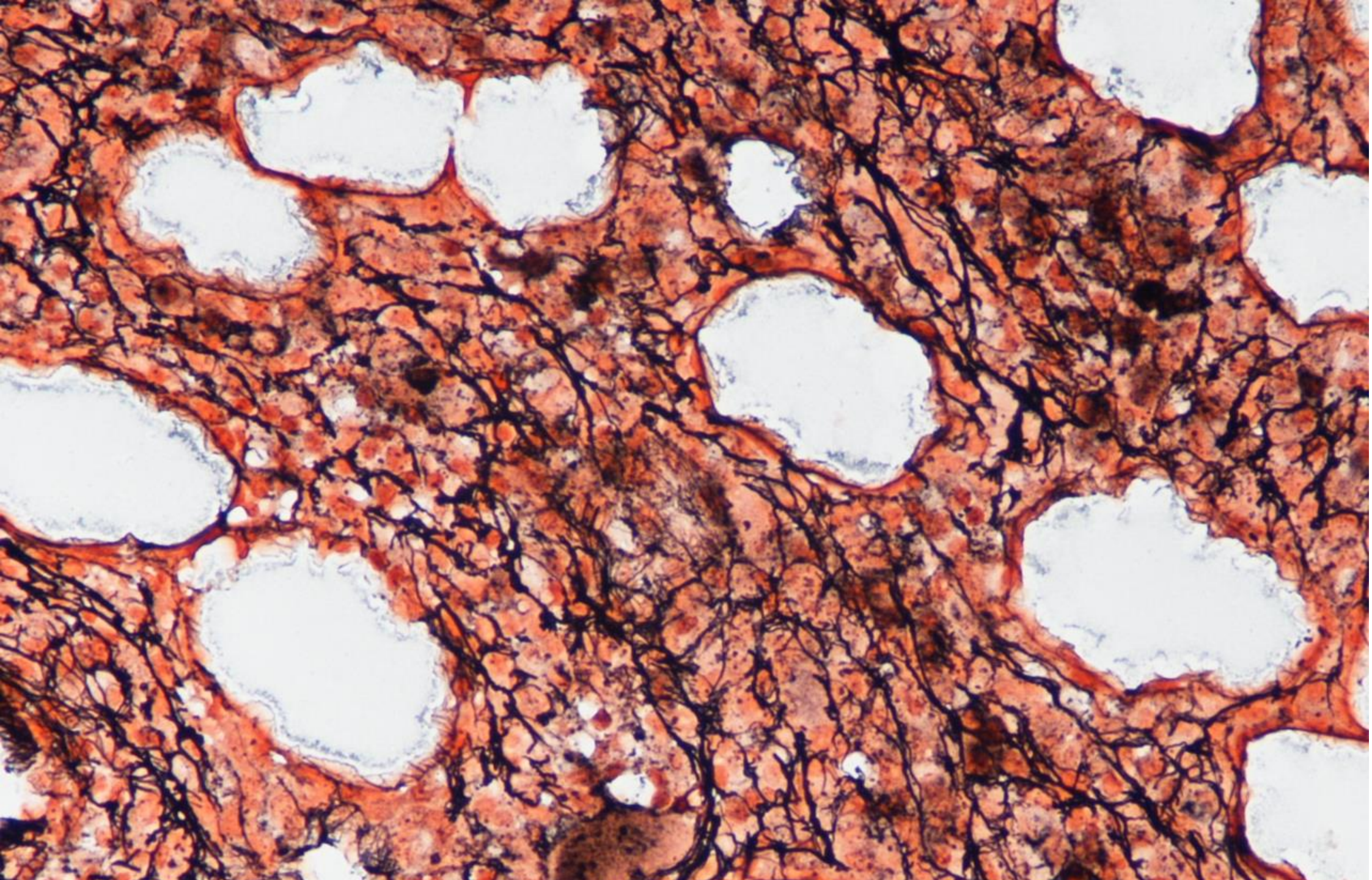

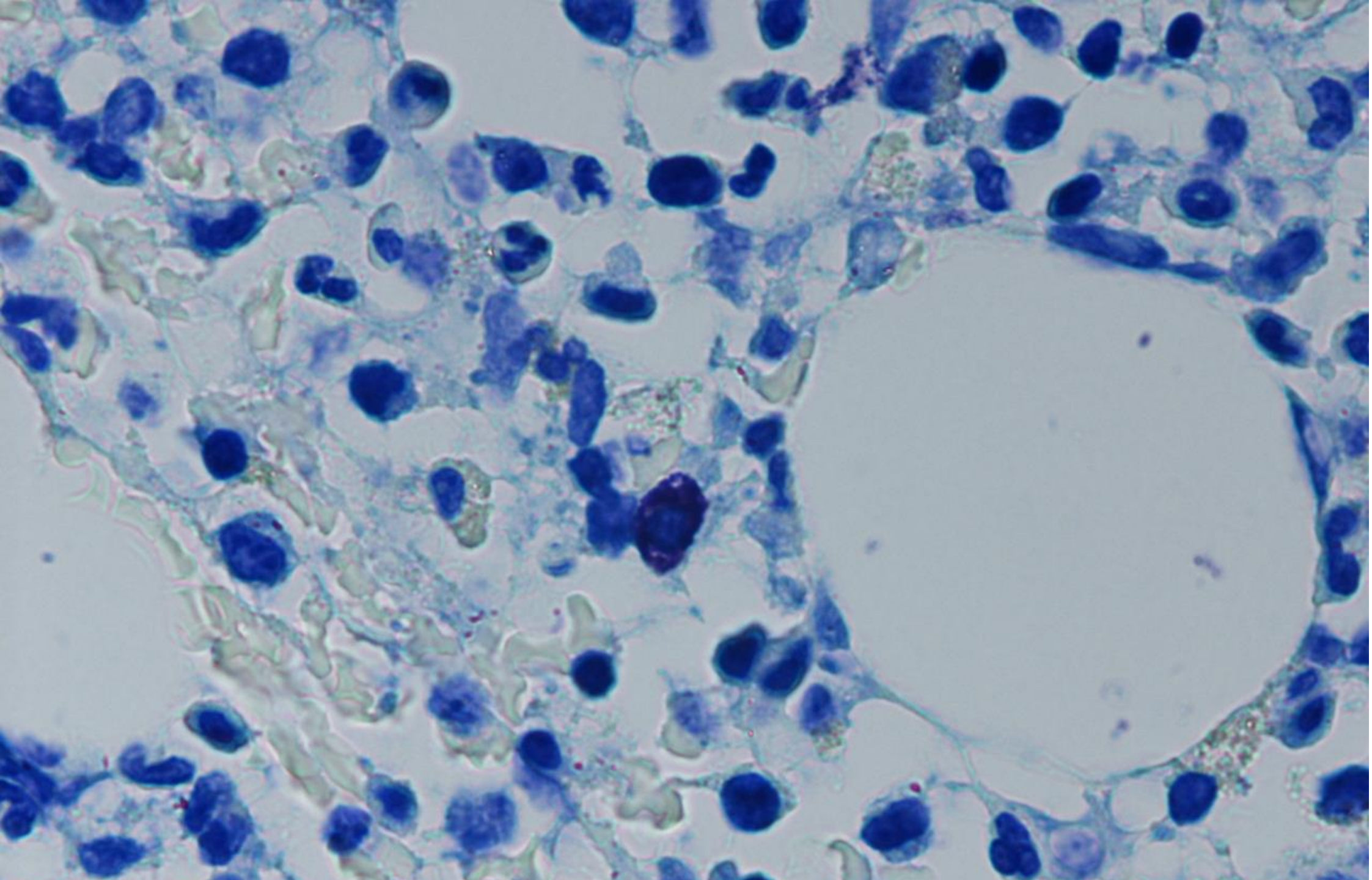

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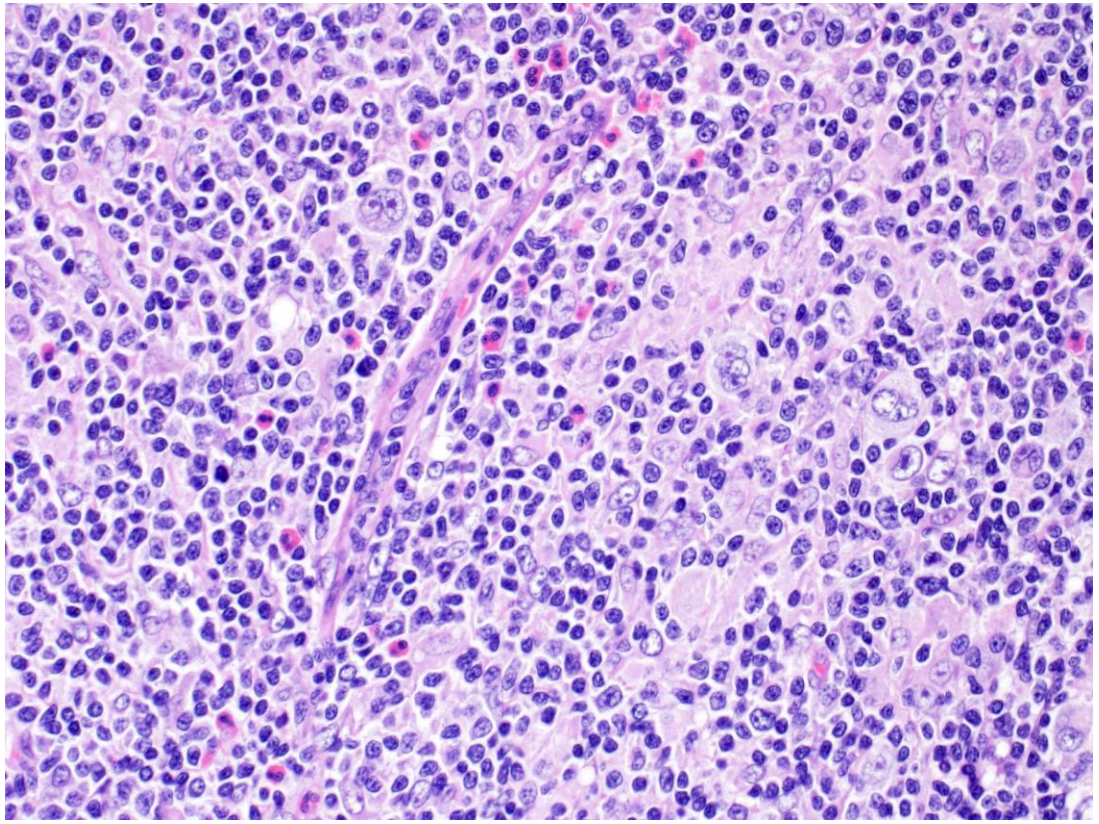
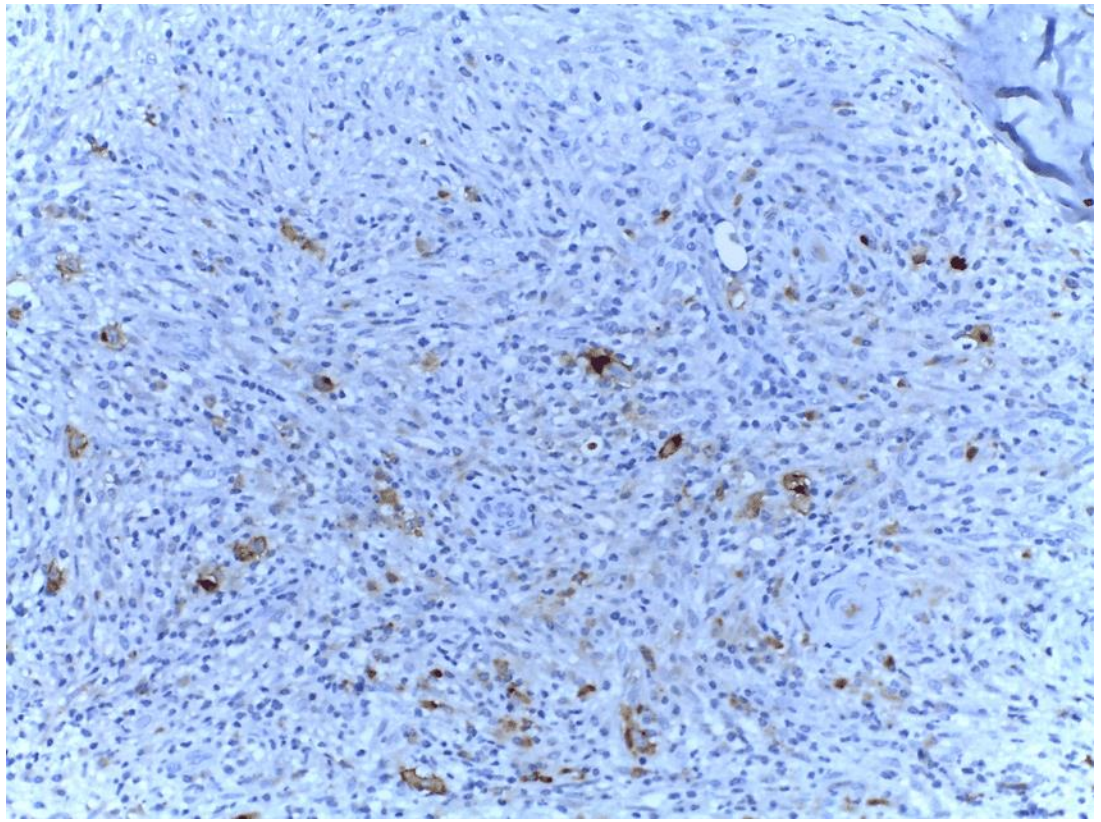
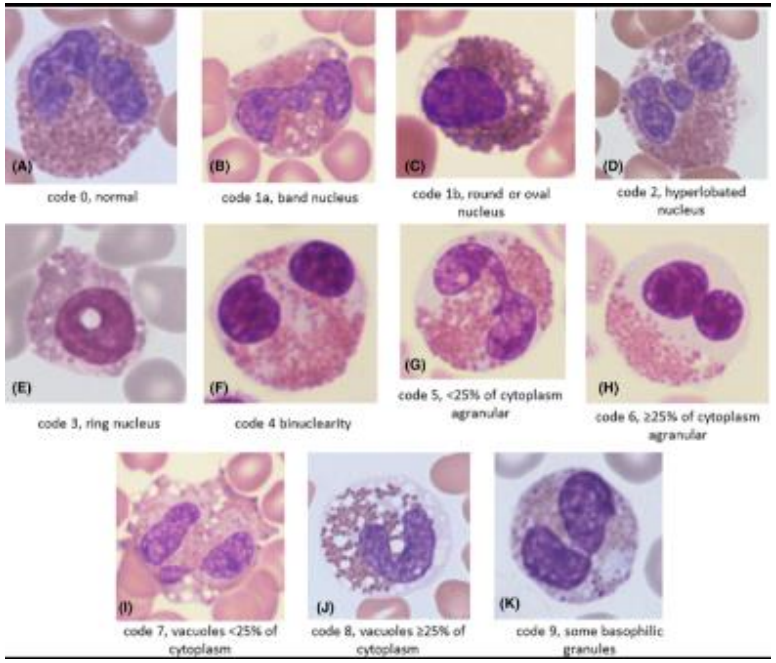
Hodgkin Lymphoma

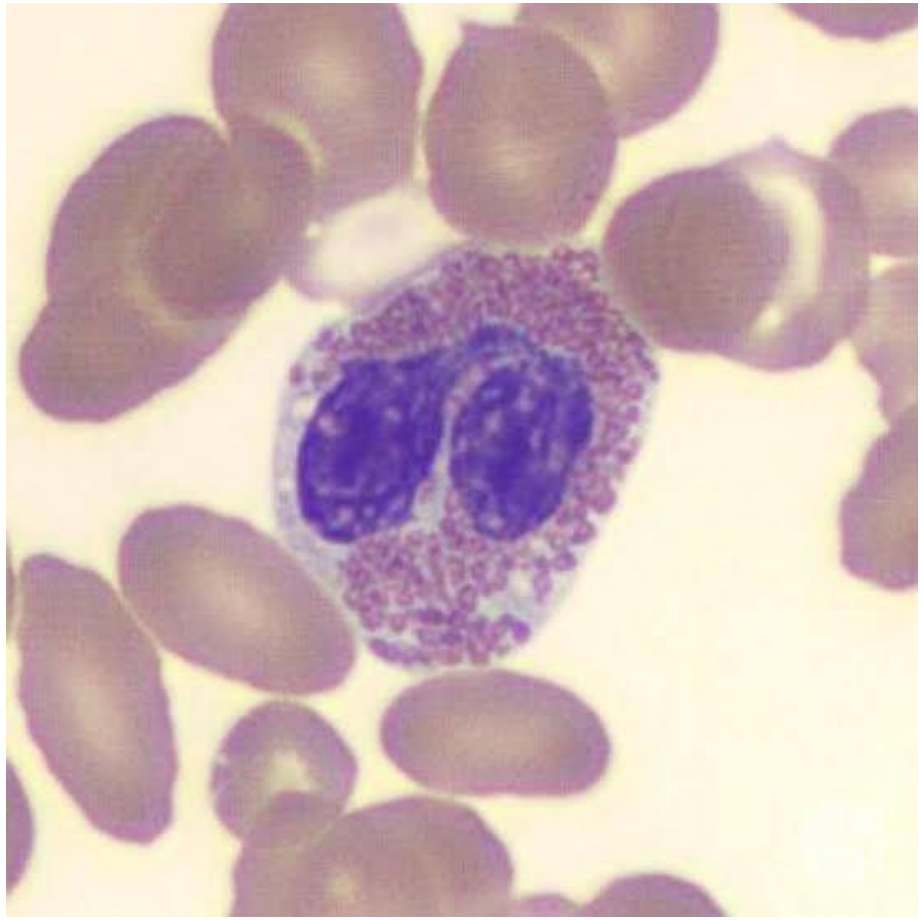




Jean Guasguen, John Bennett, Barbara Bain et al. The Role of eosinophil morphology in distinguishing between a reactive eosinophilia and eosinophilia as a feature of a myeloid neoplasm. BJH 2020, **191**, 497-504

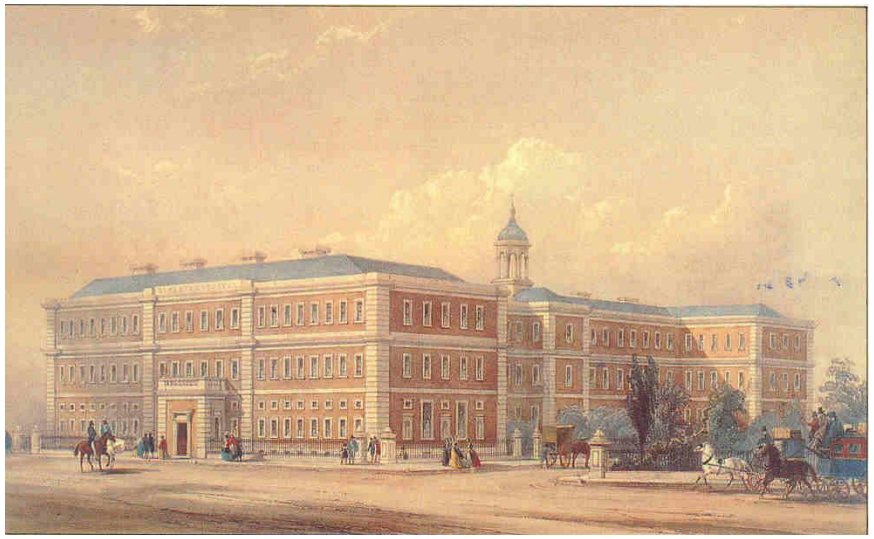


The End



My Favourite Cell – the Spherocyte

Barbara J Bain

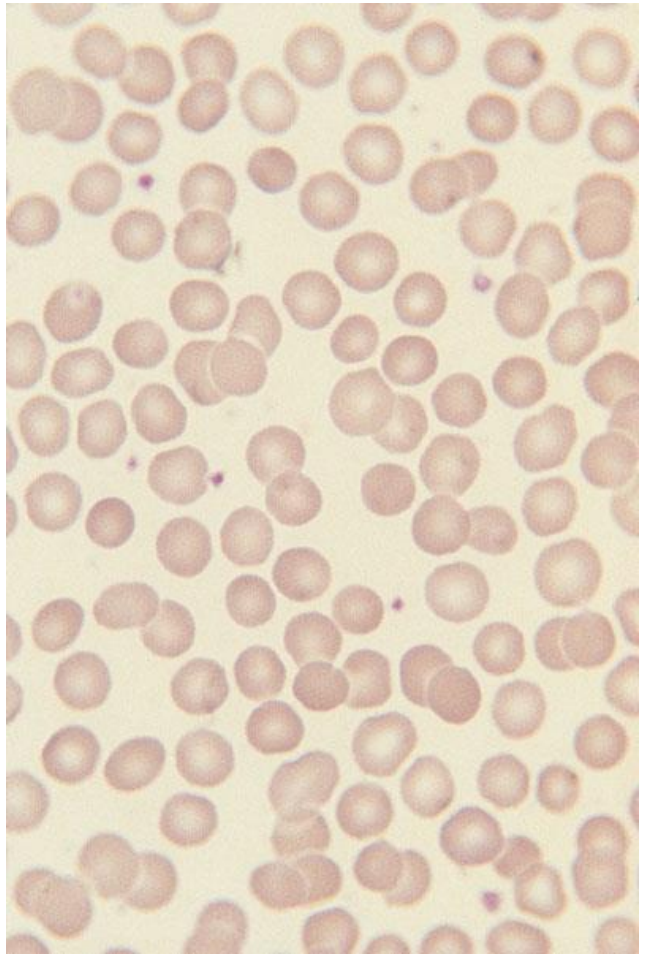


The spherocyte

- Is it a spherocyte? (examine the right part of the film)
- Is it an irregularly contracted cell?
- Is it a microspherocyte/spheroschistocyte?
- If it is a spherocyte, why has it happened?

The spherocyte

- It is a spherocyte — hereditary spherocytosis



The spherocyte

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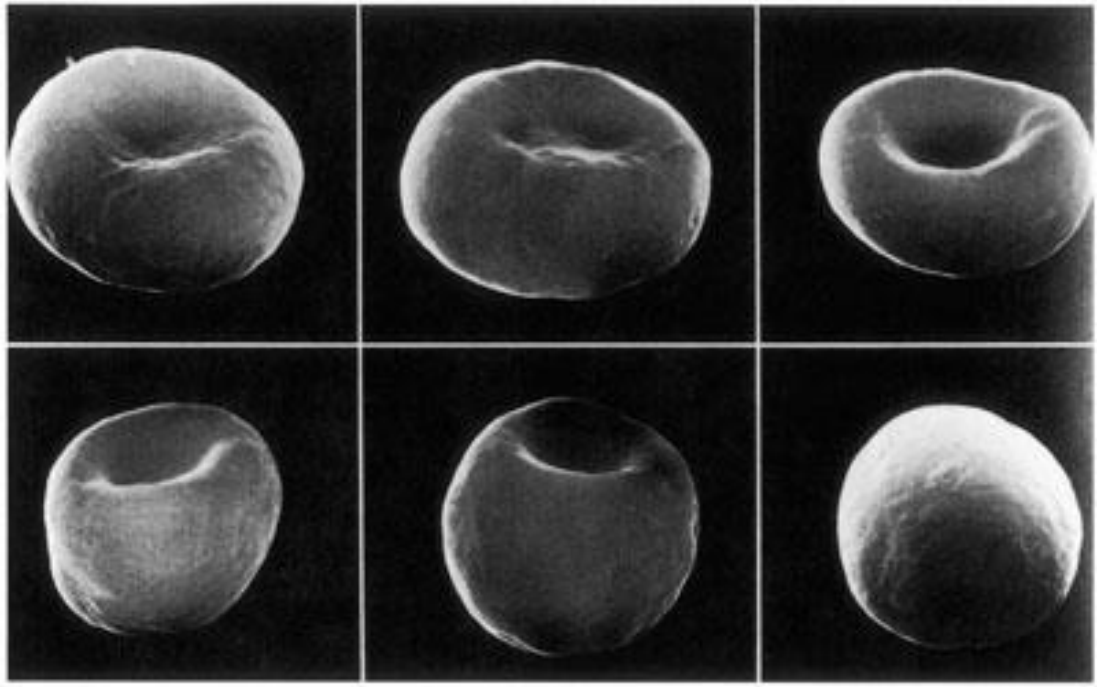
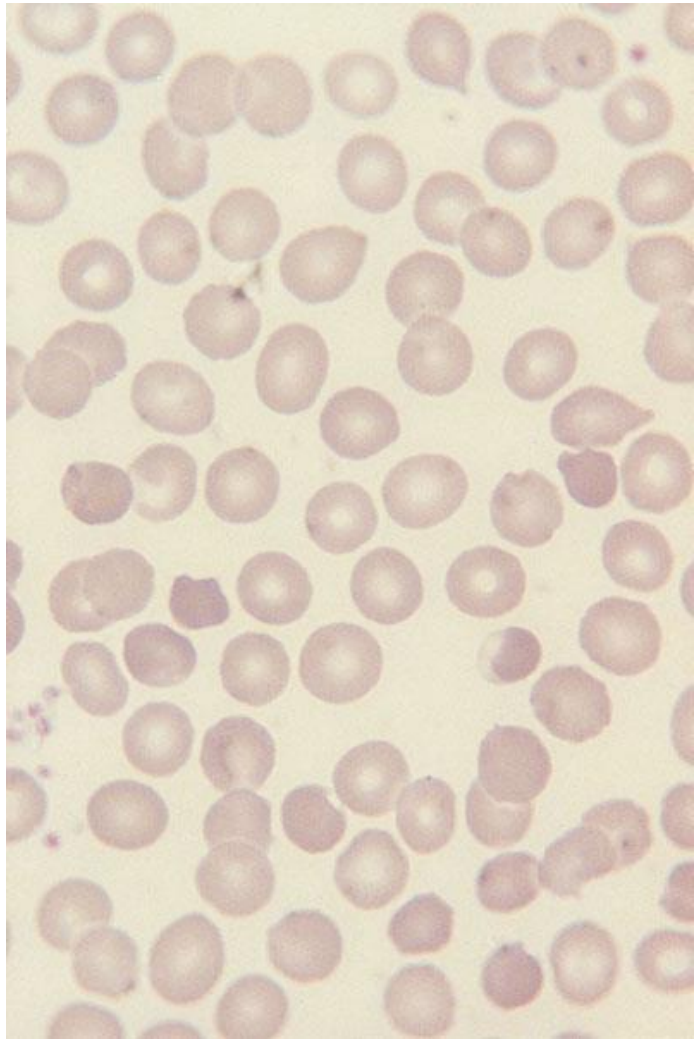


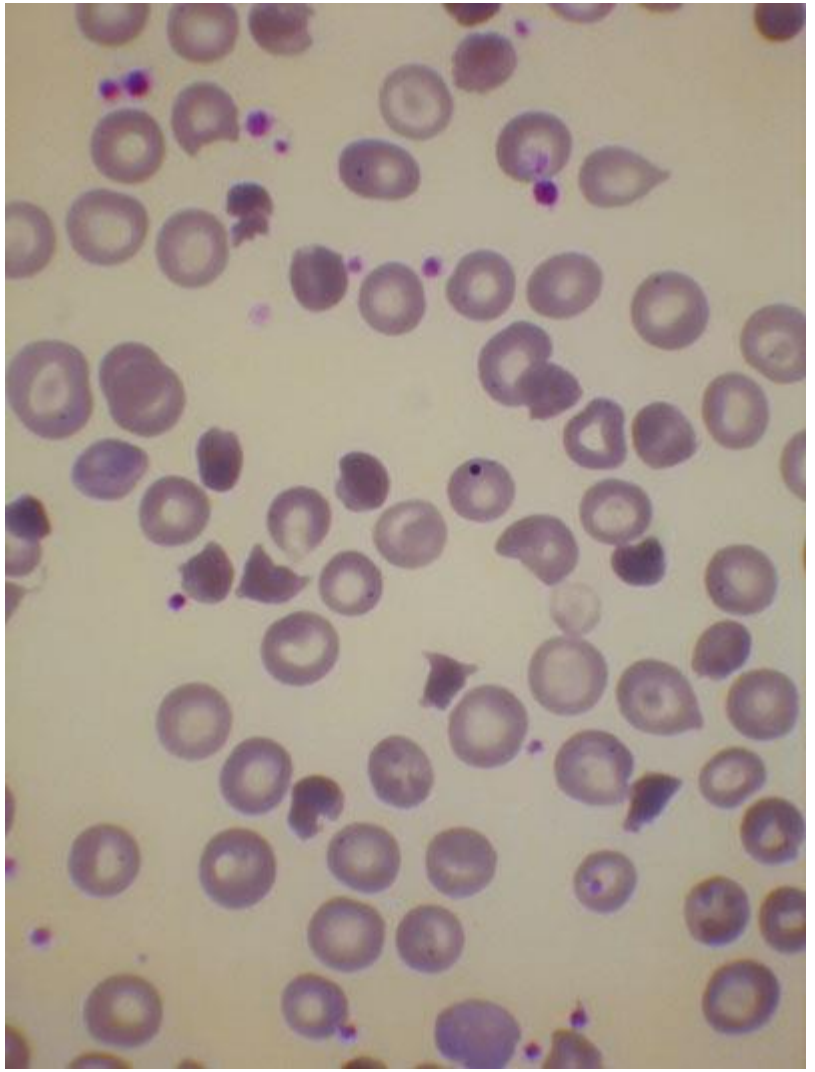
Not a spherocyte

- Not a spherocyte — irregularly contracted cells due to haemoglobin Köln



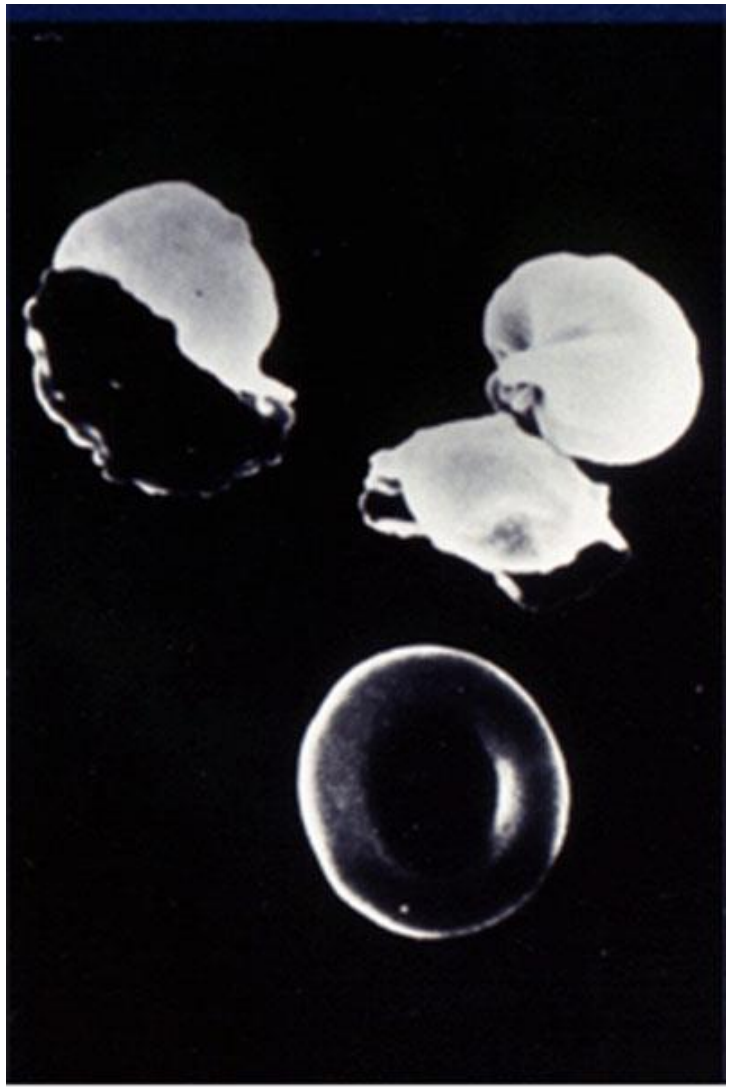
Not a spherocyte

- Not a spherocyte — irregularly contracted cells due to glutathione peroxidase deficiency



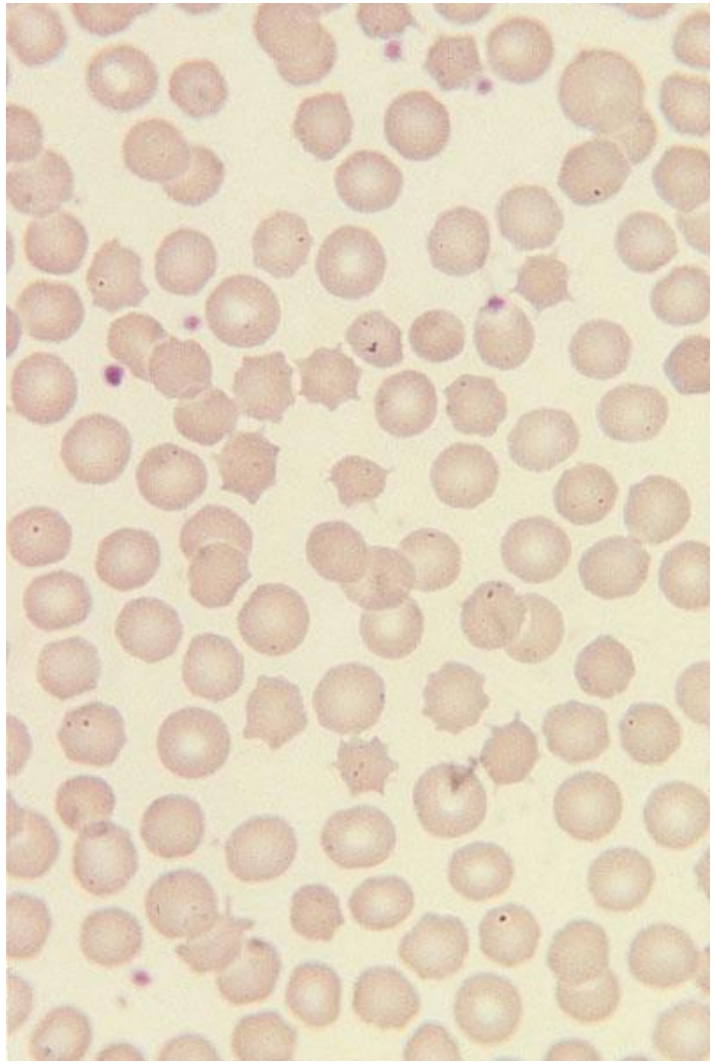
Not a spherocyte

- Irregularly contracted cells due to G6PD deficiency



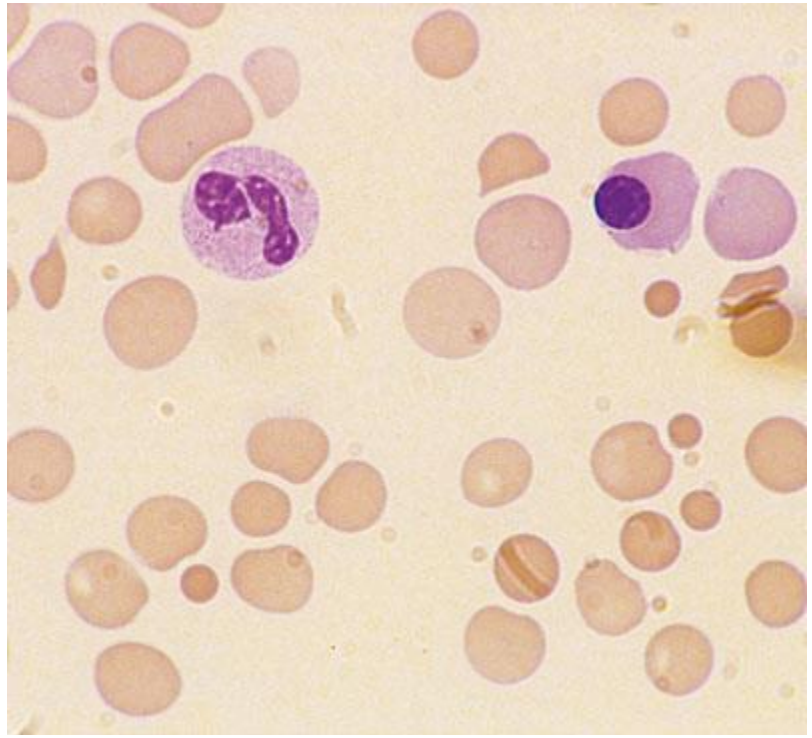
Not exactly a spherocyte

- HS, post-splenectomy — spheroacanthocytes



The spherocyte

- Microangiopathic haemolytic anaemia — microspherocytes

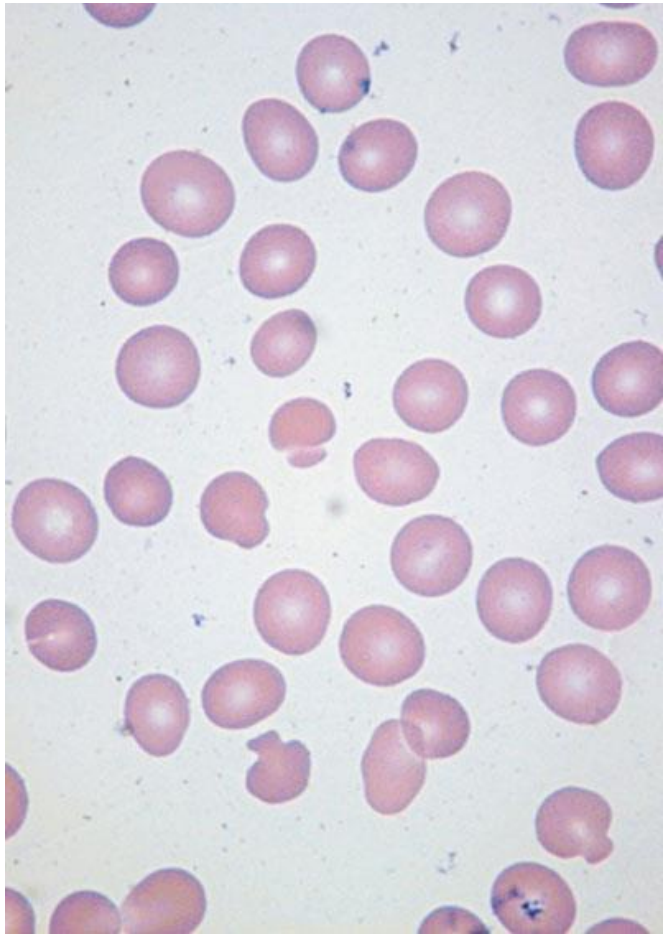


The spherocyte

- If it is a spherocyte, what is the mechanism?
  - Intrinsically abnormal red cell membrane
  - Damage to membrane by an antibody
  - Damage to membrane by a toxin or heat

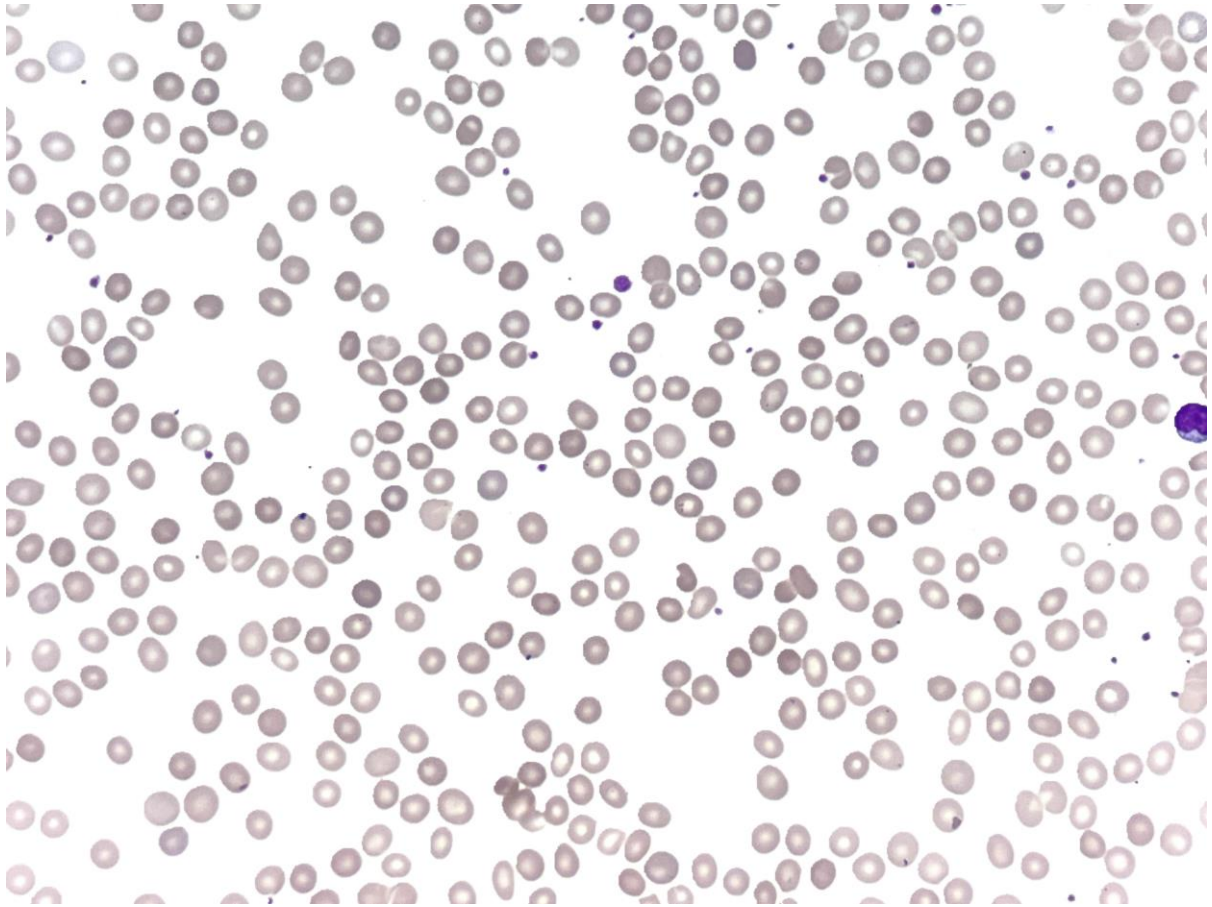
The spherocyte

- Hereditary spherocytosis due to band 3 deficiency



A genetically unusual spherocytosis

- Anaemia
- Spherocytes
- Occasional oval cells and teardrops



A genetically unusual spherocytosis

- The most common forms of hereditary spherocytosis are autosomal dominant with mutations in
  - *ANK1* 50% of cases
  - *SPTB* 30%
  - *SLC4A1* 15-20%

A genetically unusual spherocytosis

- This case is unusual as it is autosomal recessive and due to a mutation in *SPTA1*
- This causes spherocytosis only in homozygosity, compound heterozygosity or when coinherited with a low expression allele

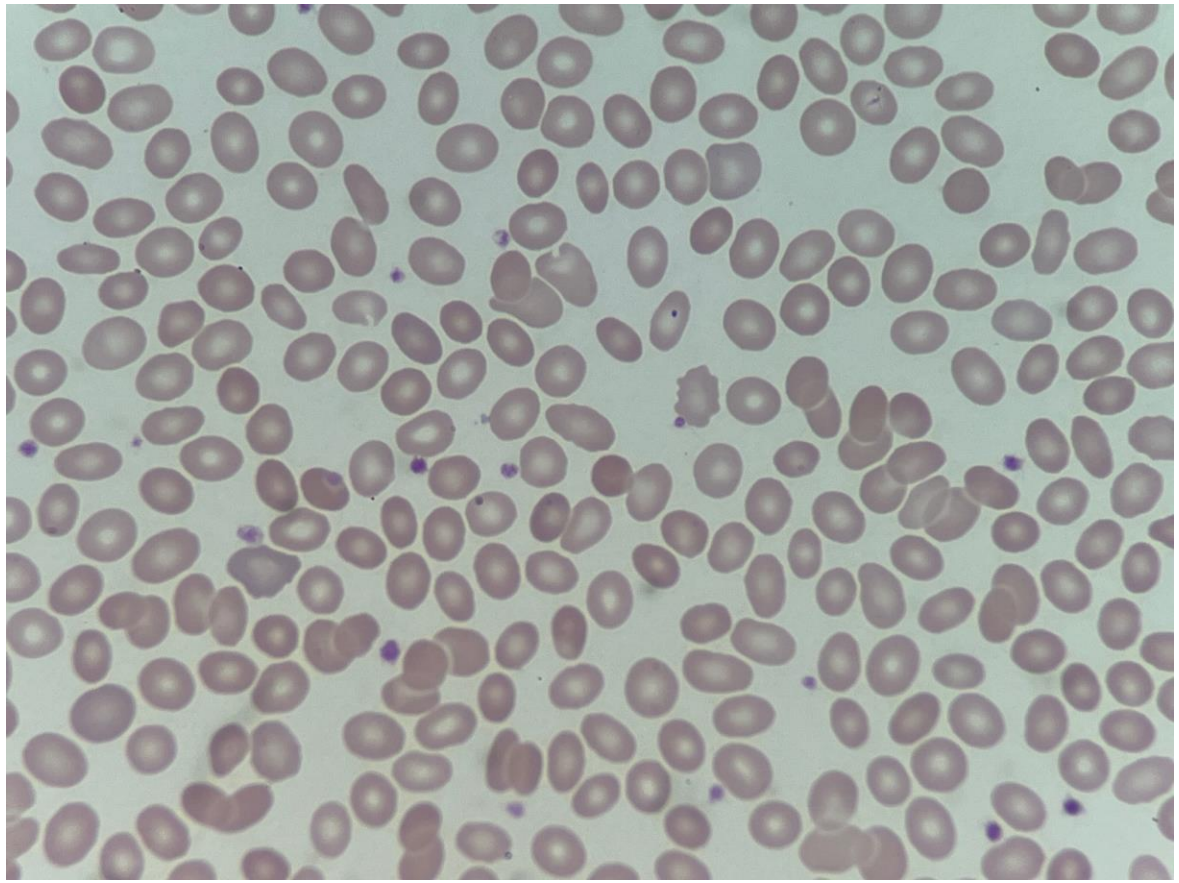
A genetically unusual spherocytosis

- In this patient there was coinheritance of an *SPTA1* mutation and α spectrin^{LELY}
- Of interest the EMA binding was repeatedly normal
- Normal EMA binding does not exclude the diagnosis, which was confirmed by morphology plus genetic analysis

Molina-Arrebola M-A and Bain BJ (2025) Hereditary spherocytosis due to an *SPTA1* nonsense mutation coinherited with α spectrin^{LELY} in *trans. Am J Hematol*, in press

An even more unusul haemolytic anaemia with some spherocytes

- Spherocytes and elliptocytes
- Intermittent anaemia needing transfusion



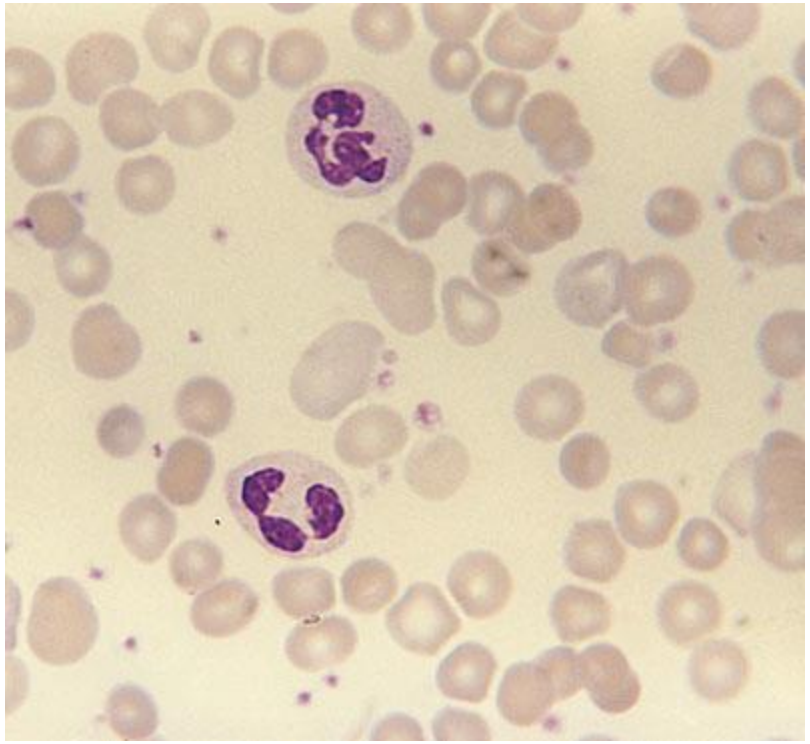
An even more unusul haemolytic anaemia with some spherocytes

- EMA binding abnormal
- This patient had a condition that is sometimes called spherocytic elliptocytosis and sometimes hereditary elliptocytosis
- She was heterozygous for β spectrin Tandil

Molina-Arrebola M-A and Bain BJ (2025) Hereditary elliptocytosis resulting from heterozygosity for β spectrin Tandil. *Am J Hematol*, **9**, 1629–1630.

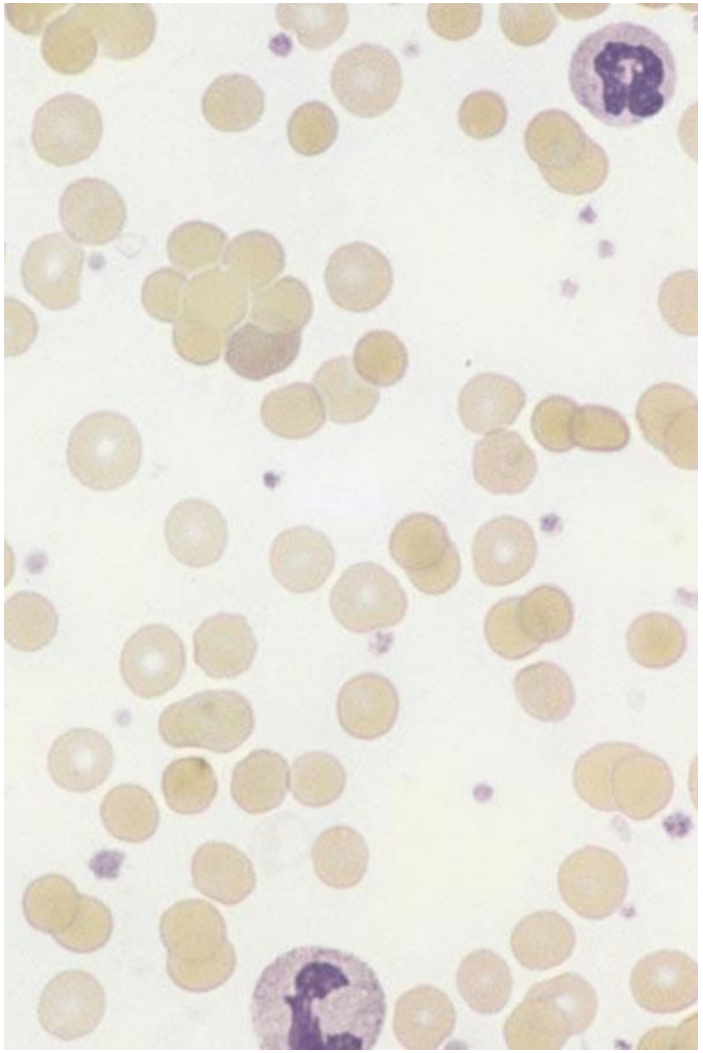
The spherocyte — immune aetiology

- Autoimmune haemolytic anaemia



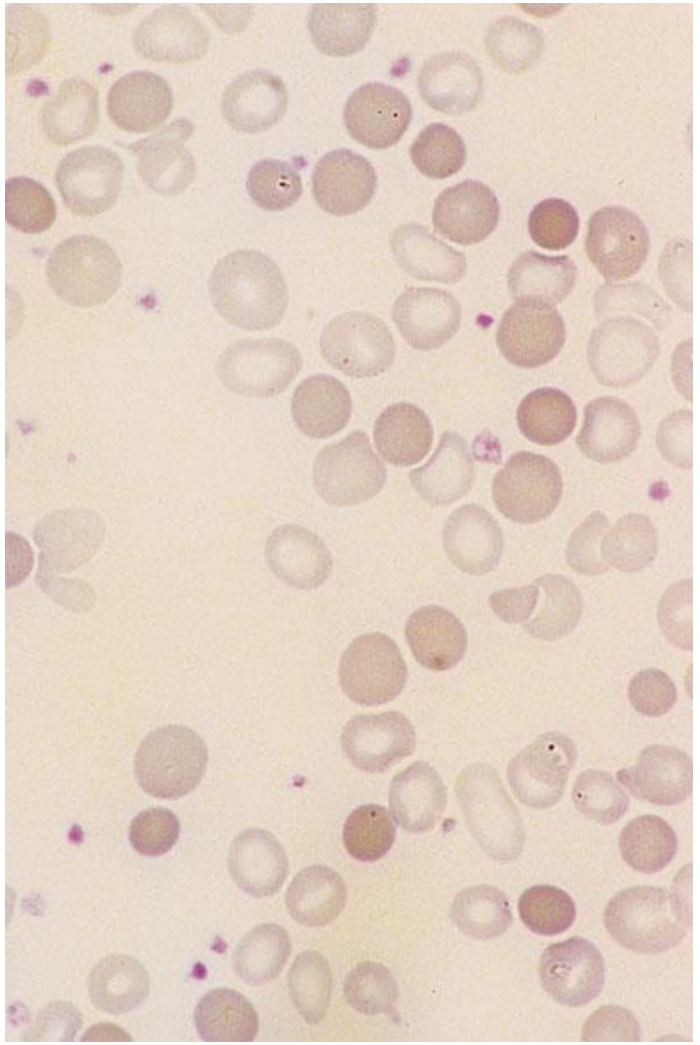
The spherocyte — immune aetiology

- Paroxysmal cold haemoglobinuria



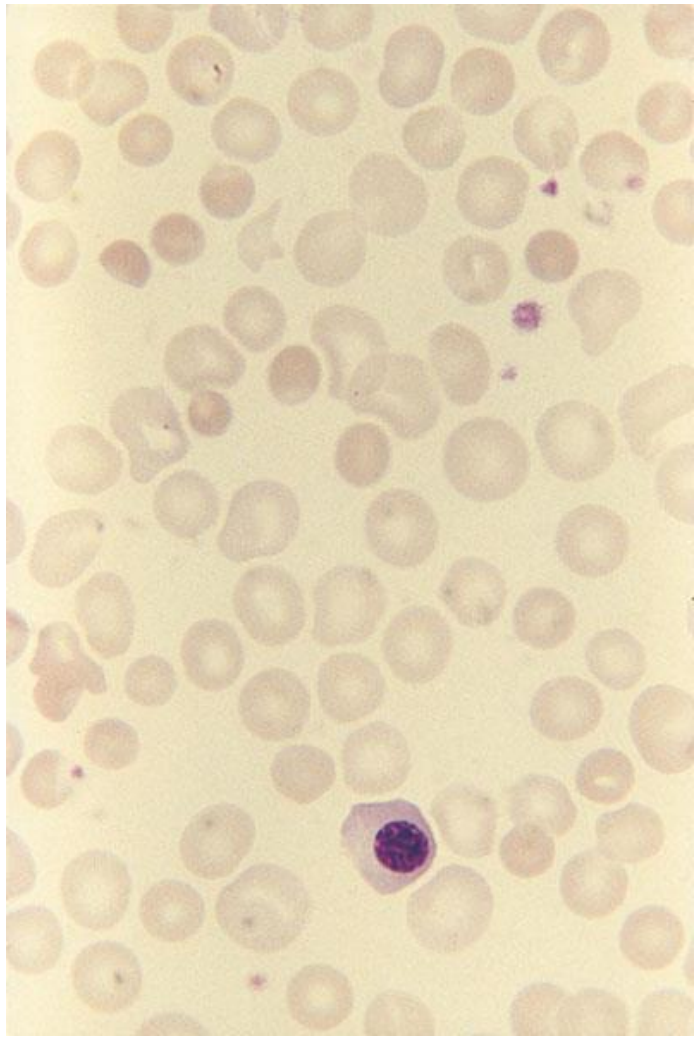
The spherocyte — immune aetiology

- Transfusion of D-positive cells into a D-negative patient



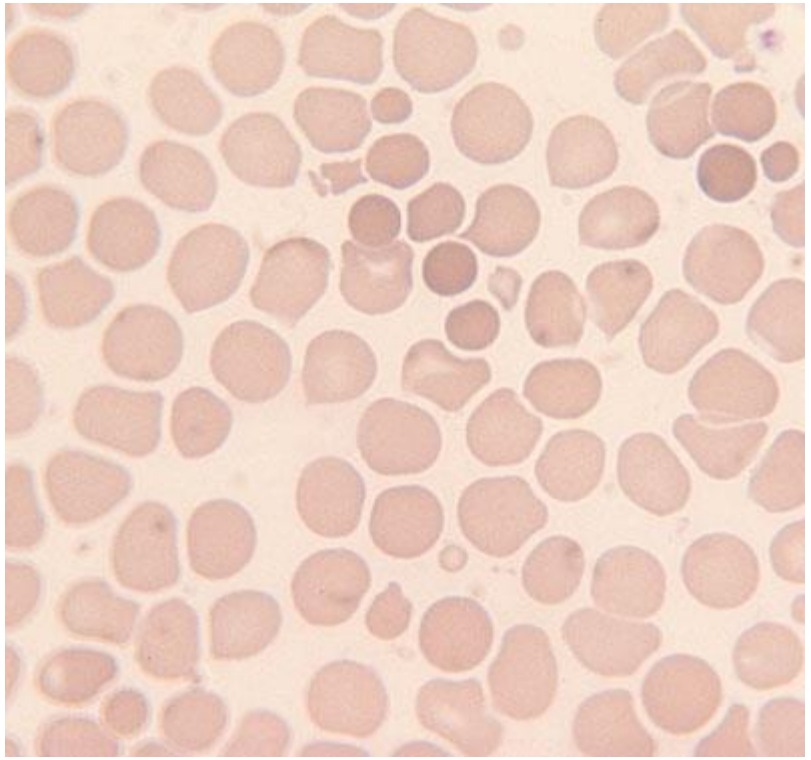
The spherocyte — immune aetiology

- ABO haemolytic disease of the newborn



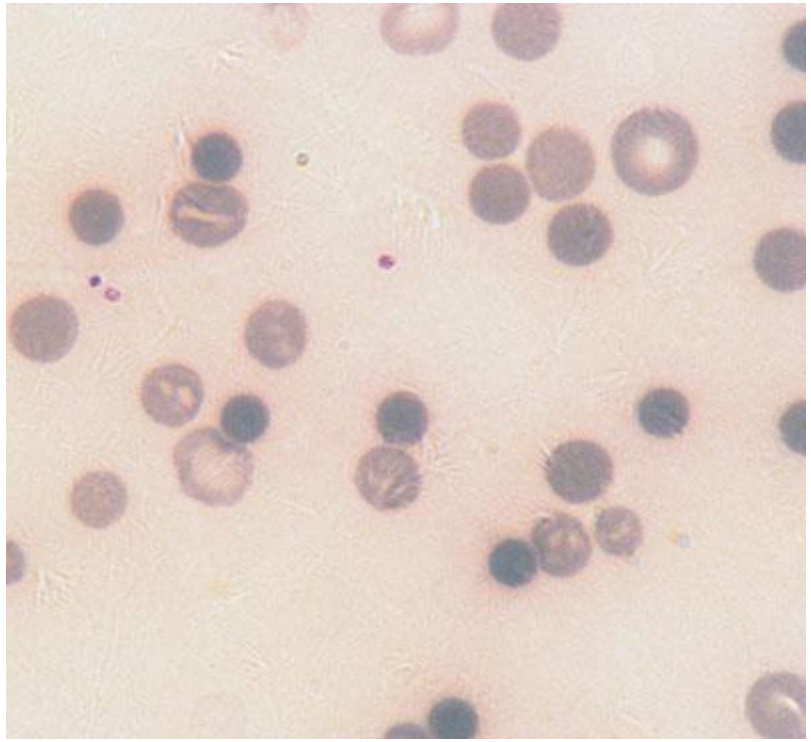
The spherocyte — damage to the membrane

- Microspherocytes in burns



The spherocyte — damage to the membrane

- *Clostridum perfringens*



Conclusions

- Ask yourself two questions
- Is it a spherocyte?
- What is the cause?

The End

Their red cells are elliptical and nucleated

NHS North Midlands and Cheshire Pathology Service

University Hospitals of North Midlands NHS Trust

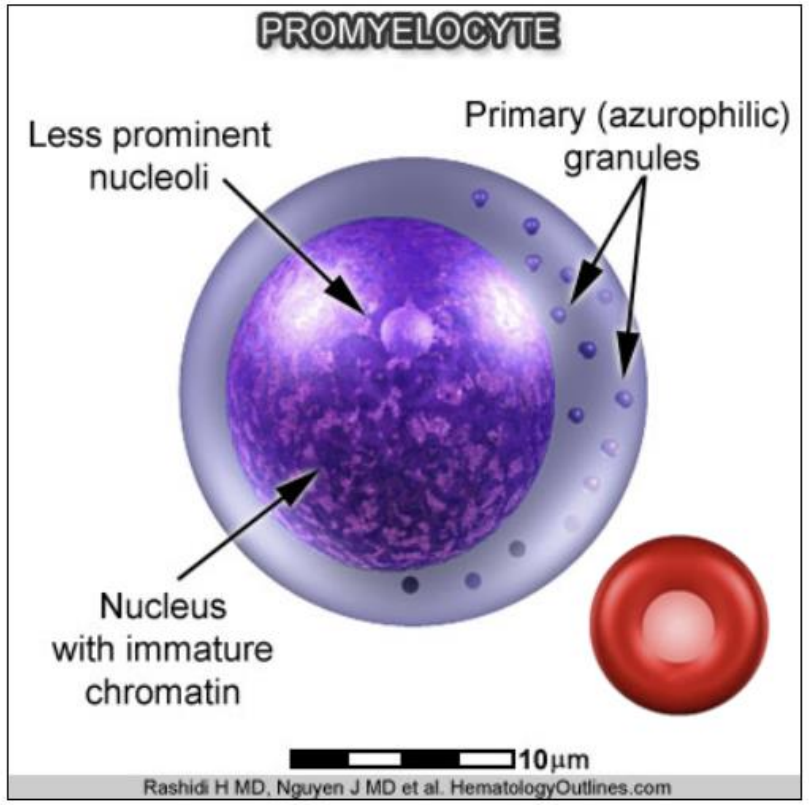
SUPERHERO OR VILLAIN

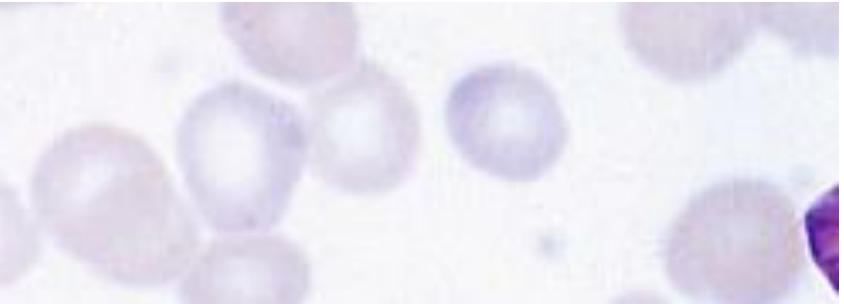
Nicki Lawrence

Principal Biomedical Scientist

Advanced Practitioner in Morphology

NMCPS, University Hospital of North Midlands NHS Trust, Stoke on Trent



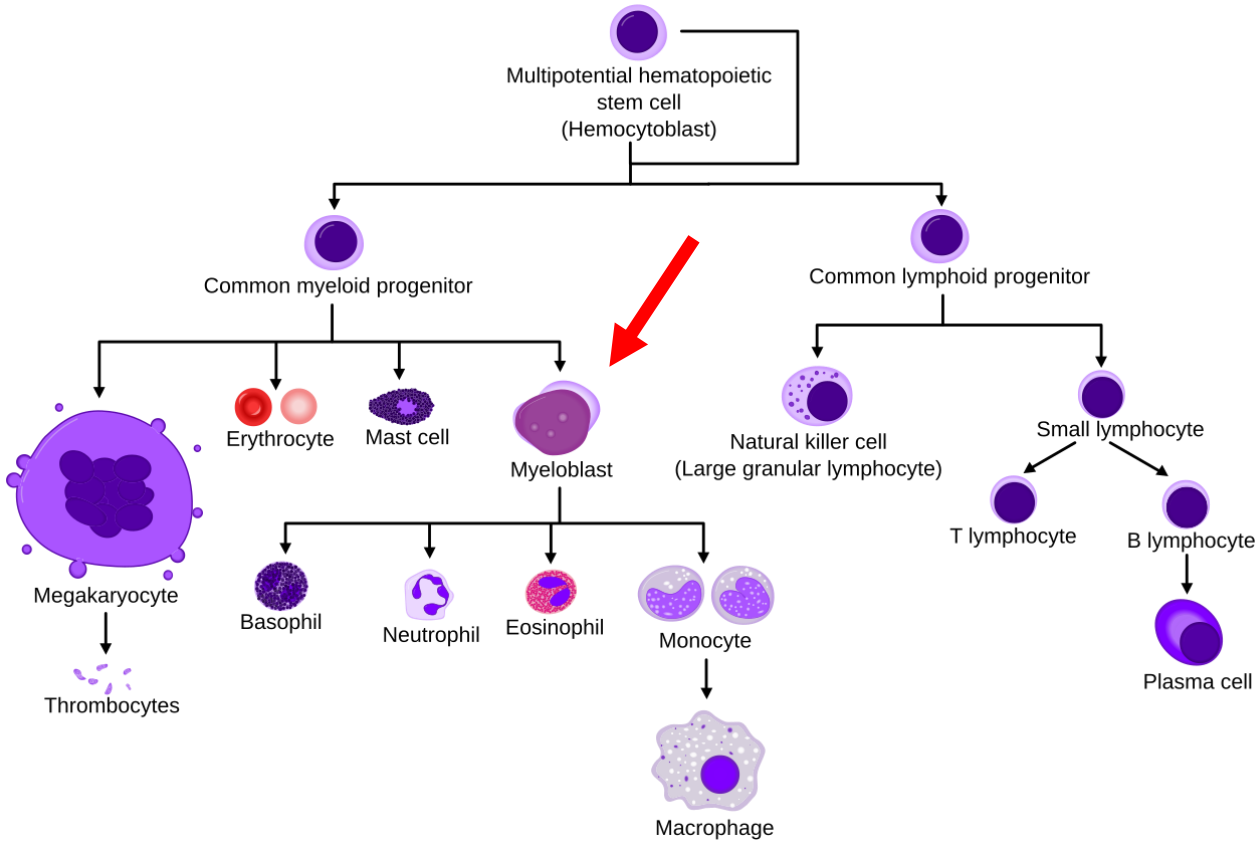


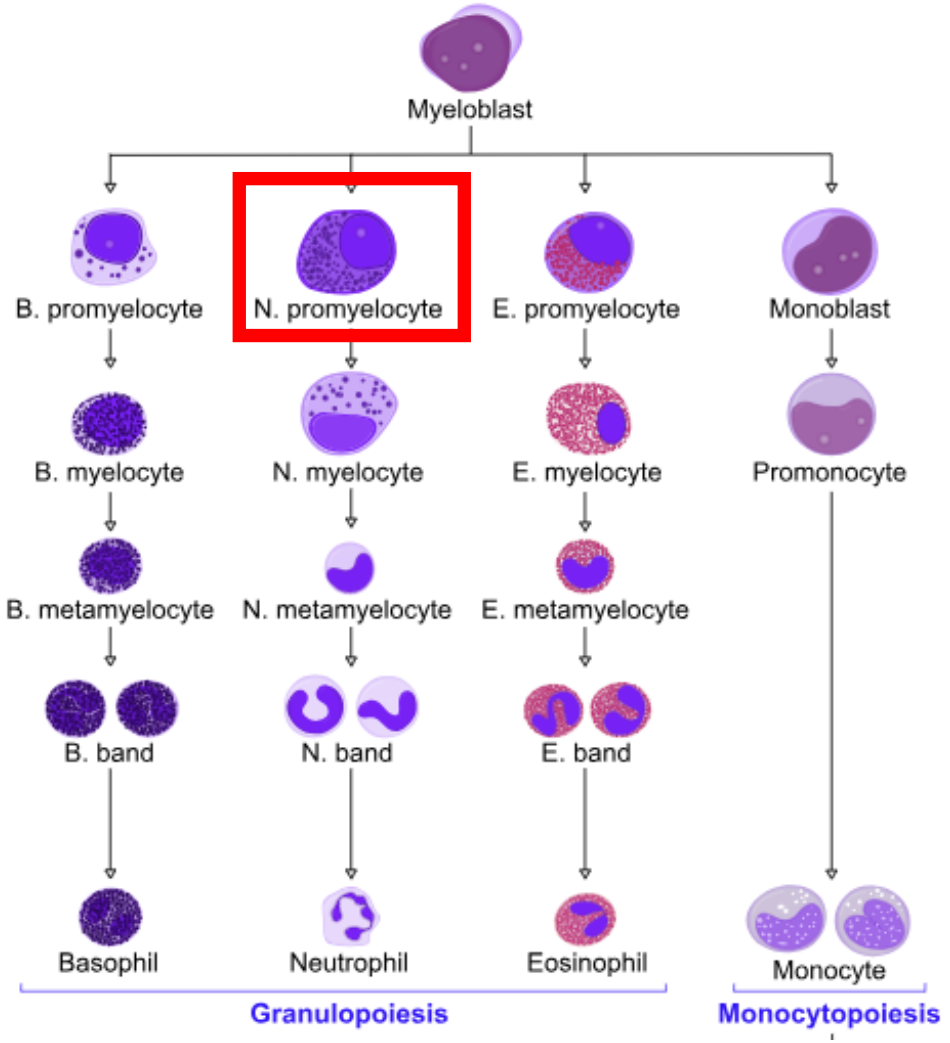
SUPERHERO OR VILLAIN?

- Promyelocytes are larger than myeloblasts and measure approximately 12–20 microns in diameter
- The nucleus of a promyelocyte is approximately the same size as a myeloblast but their cytoplasm is much more abundant
- The cytoplasm is basophilic and contains primary red/purple granules
- A Golgi zone may be visible as a paranuclear hof or clearing
- Promyelocytes have less prominent nucleoli than myeloblasts but they may be visible
- The nuclear chromatin is often described as finely dispersed but it is slightly coarser than a myeloblast
- Promyelocytes comprise approximately 2% of nucleated cells in the bone marrow and do not circulate in peripheral blood under normal conditions

HematologyOutlines – Atlas RBC size comparison

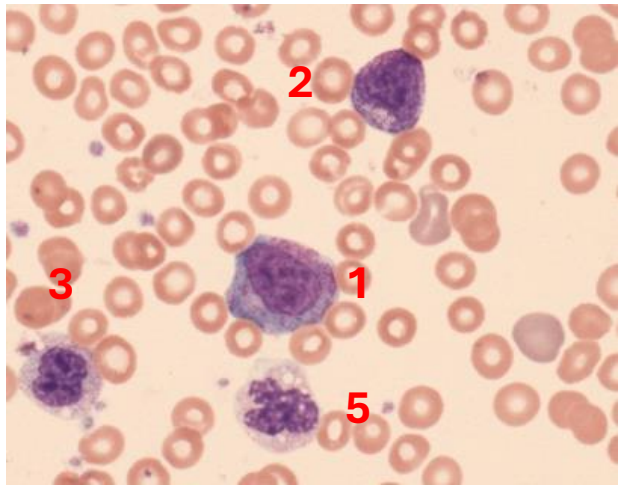
GRANULOPOIESIS





- Image: Hematopoiesis (human) diagram.png by A. Rad, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=7351905

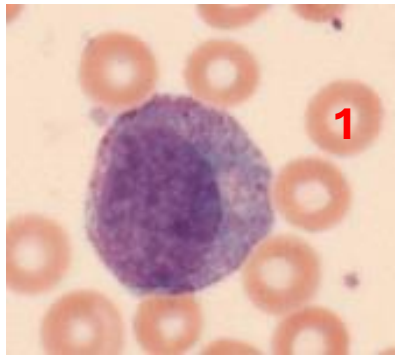
SUPERHERO

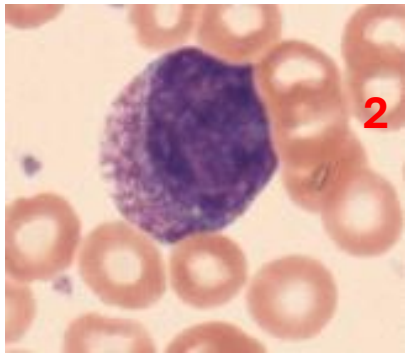


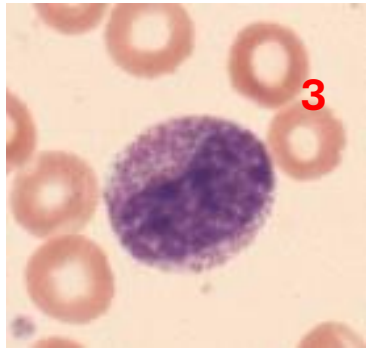
Key for all images:

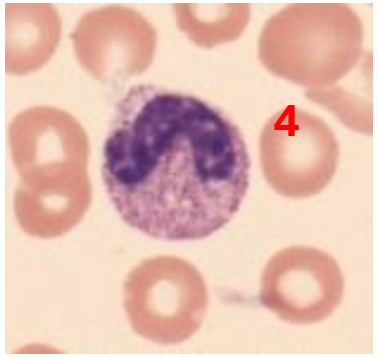
1. Promyelocyte
2. Myelocyte
3. Metamyelocyte
4. Band Form Neutrophil
5. Neutrophil
6. Toxic Vacuolated Neutrophil

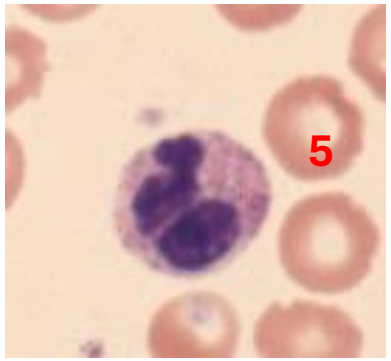
- Promyelocytes play an essential role in haematopoiesis by serving as an intermediate in the differentiation pathway leading to mature granulocytes
- Granulocytes are a vital part of the innate immune system

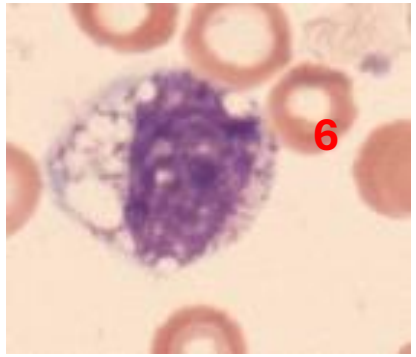












VILLAIN

Patient 1 – 69yo male, unwell, GP

14. Tests	Result	Units	Flags and Ref. Ranges	Status
Haemoglobin	79	g/L	L (120 - 170)	R
White Cell Count	1.9	10*9/L	L (4.0 - 11.0)	R
Platelets	22	10*9/L	L (150 - 450)	R
Red Cell Count	2.27	10*12/L	L (4.30 - 5.70)	R
Haematocrit	0.210		L (0.37 - 0.51)	R
Mean Cell Volume	92.3	fL	(80 - 100)	R
Mean Cell Haemoglobin	35.0	pg	H (26.5 - 31.5)	R
Mean Cell Hb Concentration	379	g/L	H (300 - 350)	R
Mean Platelet Volume	9.9	fl		R
Red cell distribution width	17.8			R
Platelet distribution width	63.6			R
% Hypochromic Red Cell	0.9	%	(<5.0)	R
Neut (abs no)	0.90	10*9/L	L (2.0 - 7.5)	R
Lymph (abs no)	0.70	10*9/L	L (1.10 - 3.60)	R

14. Tests	Result	Units	Flags and Ref. Ranges	Status
>Mono (abs no)	0.00	10*9/L	L (0.20 - 0.80)	R
Eos (abs no)	0.20	10*9/L	(0.04 - 0.40)	R
Bas (abs no)	0.00	10*9/L	(0.0 - 0.10)	R
LUCs (abs no)	0.00	10*9/L	(0.00 - 0.44)	R
Neutrophils (%)	49	%	(40 - 75)	R
Lymphocytes (%)	36	%		R
Monocytes (%)	1	%	L (2 - 10)	R
Eosinophils (%)	10	%	H (1 - 6)	R
Basophils (%)	2	%	H (0 - 1)	R
Large unstained cells (%)	3	%		R
Blast Flag	+++			R
Atypical Lymph Flag				
Myelo-peroxidase Def. Flag				
Blood film	Blood film examined			R

INR	1.2
D-dimer	4428 ng/mL
Fibrinogen	2.65 g/L

Patient 2 – 33yo male, bruising, A&E

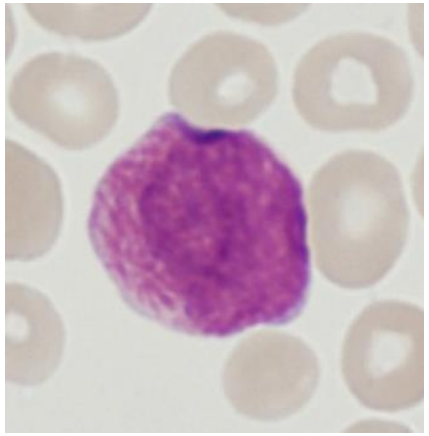
Haemoglobin	111	g/L	L (120 - 170)	R
White Cell Count	54.3	10*9/L	H (4.0 - 11.0)	R
Platelets	34	10*9/L	L (150 - 450)	R
Red Cell Count	3.27	10*12/L	L (4.30 - 5.70)	R
Haematocrit	0.299		L (0.37 - 0.51)	R
Mean Cell Volume	91.6	fL	(80 - 100)	R
Mean Cell Haemoglobin	34.0	pg	H (26.5 - 31.5)	R
Mean Cell Hb Concentration	371	g/L	H (300 - 350)	R
Mean Platelet Volume	9.9	fl		R

14. Tests	Result	Units	Flags and Ref. Ranges	Status
>Red cell distribution width	16.8			R
Platelet distribution width	81.1			R
% Hypochromic Red Cell	0.6	%	(<5.0)	R
Neut (abs no)	2.17	10*9/L	(2.0 - 7.5)	R
Lymph (abs no)	2.17	10*9/L	(1.10 - 3.60)	R
Mono (abs no)	0	10*9/L	L (0.20 - 0.80)	R
Eos (abs no)	0	10*9/L	L (0.04 - 0.40)	R
Bas (abs no)	0	10*9/L	(0.0 - 0.10)	R
LUCs (abs no)	0	10*9/L	(0.00 - 0.44)	R
>Large unstained cells (%)	0	%		R
Blast Flag	+++			R
Atypical Lymph Flag				
Myelo-peroxidase Def. Flag				
Urgent blood film	Blood film examined			R
Atyp Lymph (abs no)	0.00			R
Metamye (abs no)	0.00	10*9/L		R
Myelo (abs no)	0.00	10*9/L		R
Promye (abs no)	47.78	10*9/L		R
Blast (abs no)	2.17	10*9/L		R
Atypical Lymphocytes (%)	0	%		R
Metamyelocytes (%)	0	%		R
Myelocytes (%)	0	%		R
Promyelocytes (%)	88	%		R

INR	1.5
D-dimer	32078 ng/mL
Fibrinogen	1.02 g/L

APL - MORPHOLOGY

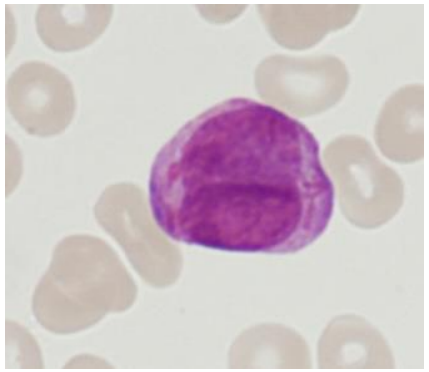
- Nuclear size and shape in the abnormal promyelocytes of hypergranular APL are irregular and greatly variable; they are often kidney shaped or bilobed



- Cytoplasm is marked by densely-packed large granules, staining bright pink, red or purple in Romanowsky stains

- Cytoplasmic granules may be so large and/or numerous that they totally obscure the nuclear cytoplasmic margin. In some cells, the cytoplasm is filled with fine dust-like granules

- Characteristic cells containing bundles of Auer rods (“faggot cells”) randomly distributed in the cytoplasm are present in most cases

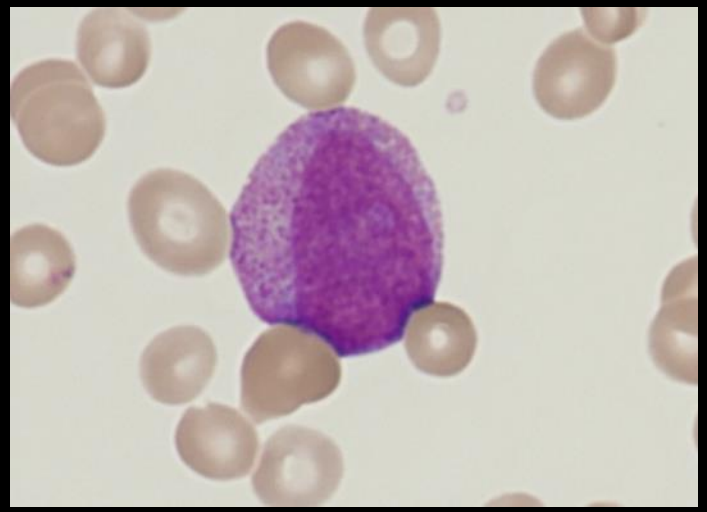


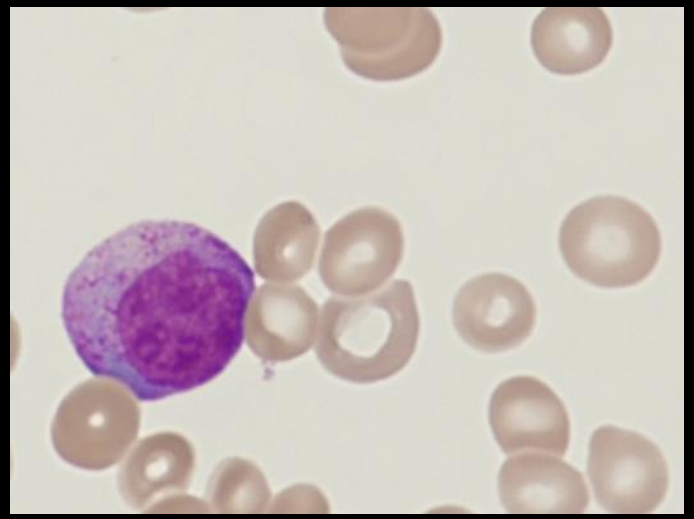
- Myeloblasts with single Auer rods may also be observed

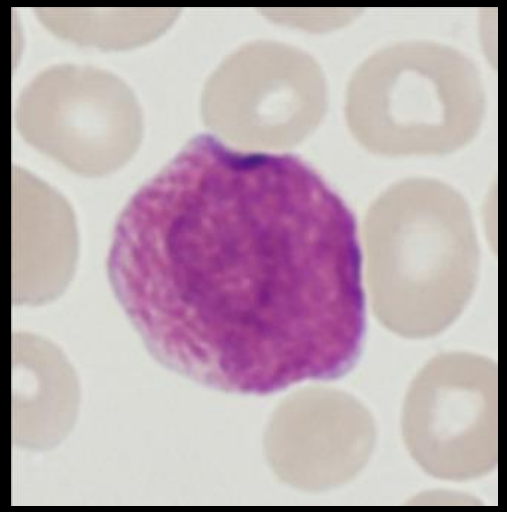
- Auer rods in hypergranular APL are usually larger than in other types of AML

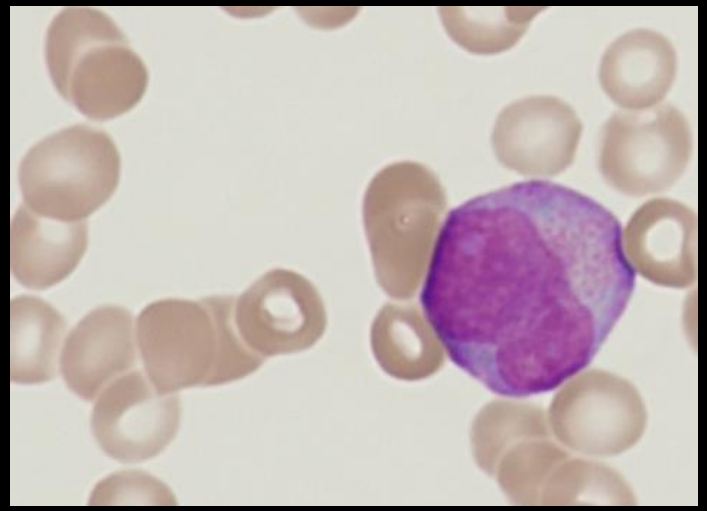
PROMYELOCYTE MORPHOLOGY PATIENT 1

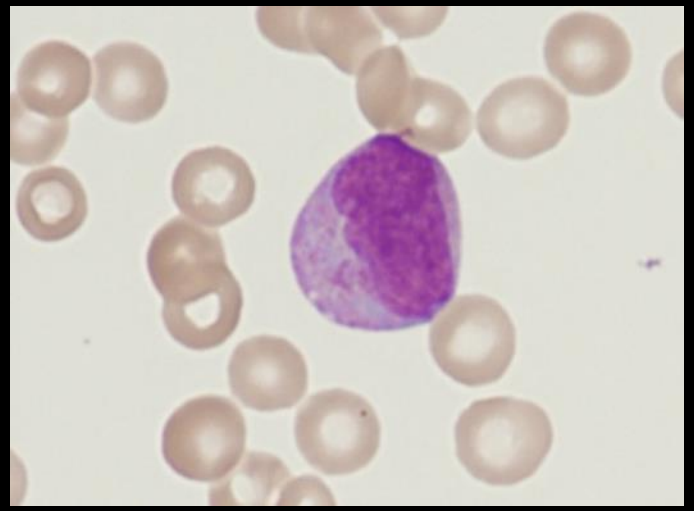
HYPERGRANULAR





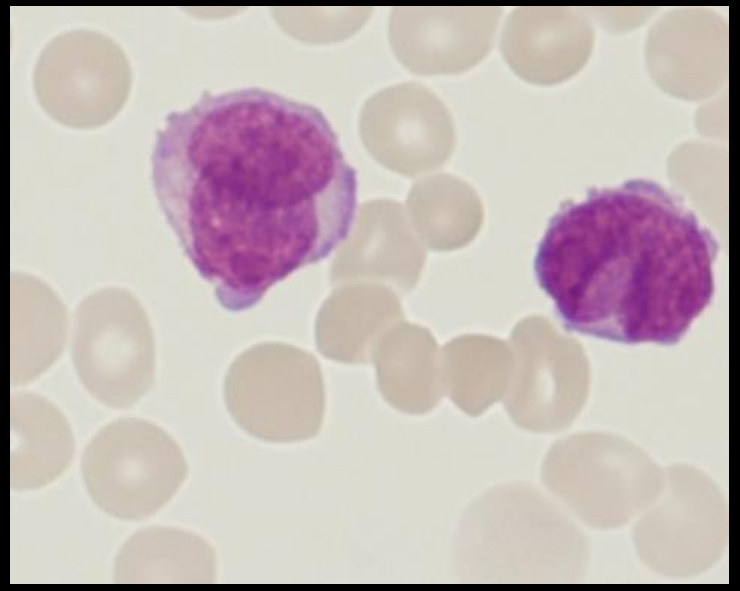


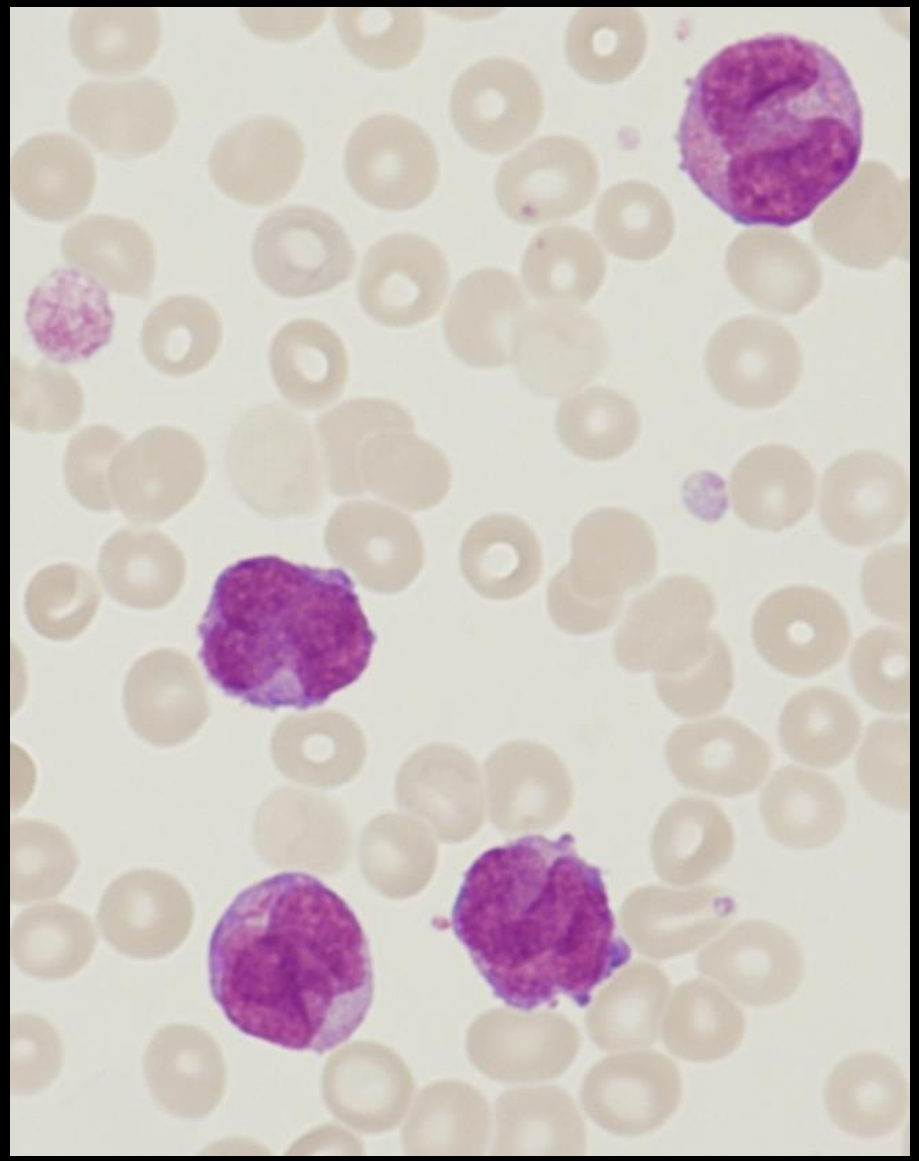


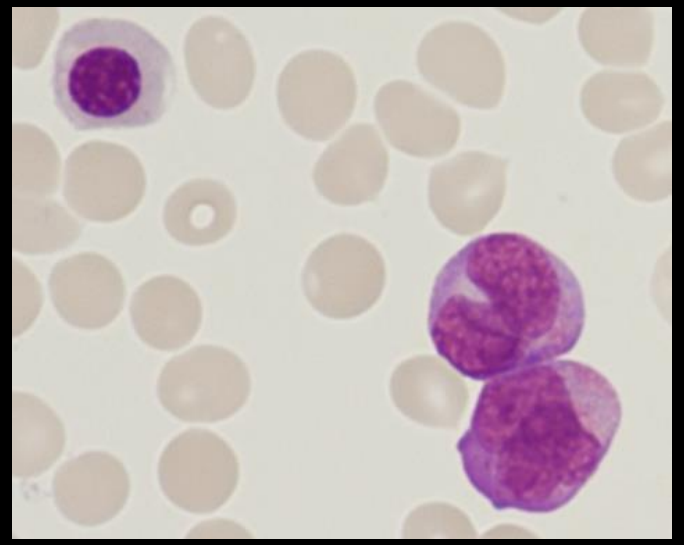


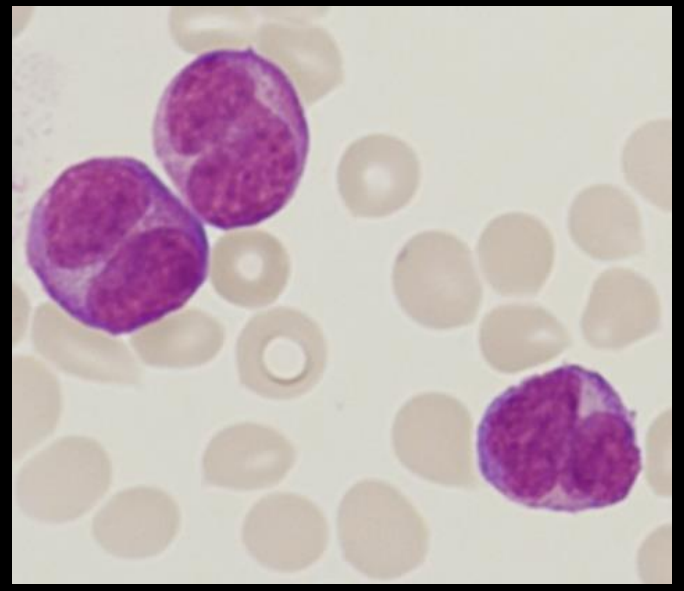
PROMYELOCYTE MORPHOLOGY PATIENT 2

MICROGRANULAR









GENETICS

PATIENT 1

Karyotype:

10 metaphase cells examined by G-band chromosome analysis

45, X-Y, t(15;17)(q24;q21), add 15 (q25), -22 (9)
46, XY (1)

PML::RARA gene rearrangement

48/50 (96%) cells had an abnormal signal pattern consistent with a *PML::RARA* gene rearrangement

Classified as Acute Promyelocytic Leukaemia with *PML::RARA* (WHO 2022)

PATIENT 2

Karyotype:
20 metaphase cells examined by G-band chromosome analysis
46,XY (20)

No evidence of a *PML::RARA* rearrangement
No evidence of a RARA gene rearrangement (Interphase FISH)

21bp and 45bp FLT3-ITDs identified
No evidence of insertion/duplication variant within the NPM1 gene
(fragment analysis)

Positive for a *PML::RARA* fusion by nested RT-PCR and by RQ-PCR

Consistent with a cytogenetically cryptic *PML::RARA* rearrangement

Classified as Acute Promyelocytic Leukaemia with *PML::RARA* (WHO 2022)

COAGULOPATHY

Lab Results

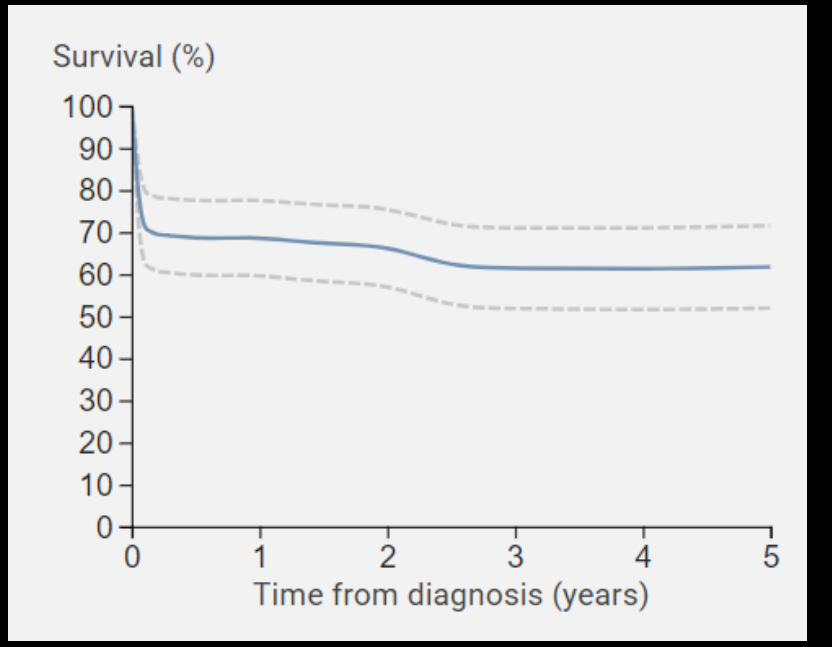
- Thrombocytopenia (<40 x10^9/L in 25%)
- Prolonged PT/APTT
- Increased D Dimer / Low fibrinogen
- Protein C, S and antithrombin not usually decreased (in contrast to DIC)
- Microvascular thrombosis uncommon
- Low platelets not solely consumptive
- Physiological anticoagulant levels preserved
- Lower fibrinogen and factor V
- D dimers higher

SUGGESTS PRIMARY DRIVER IS INCREASED FIBRINOLYTIC ACTIVTY

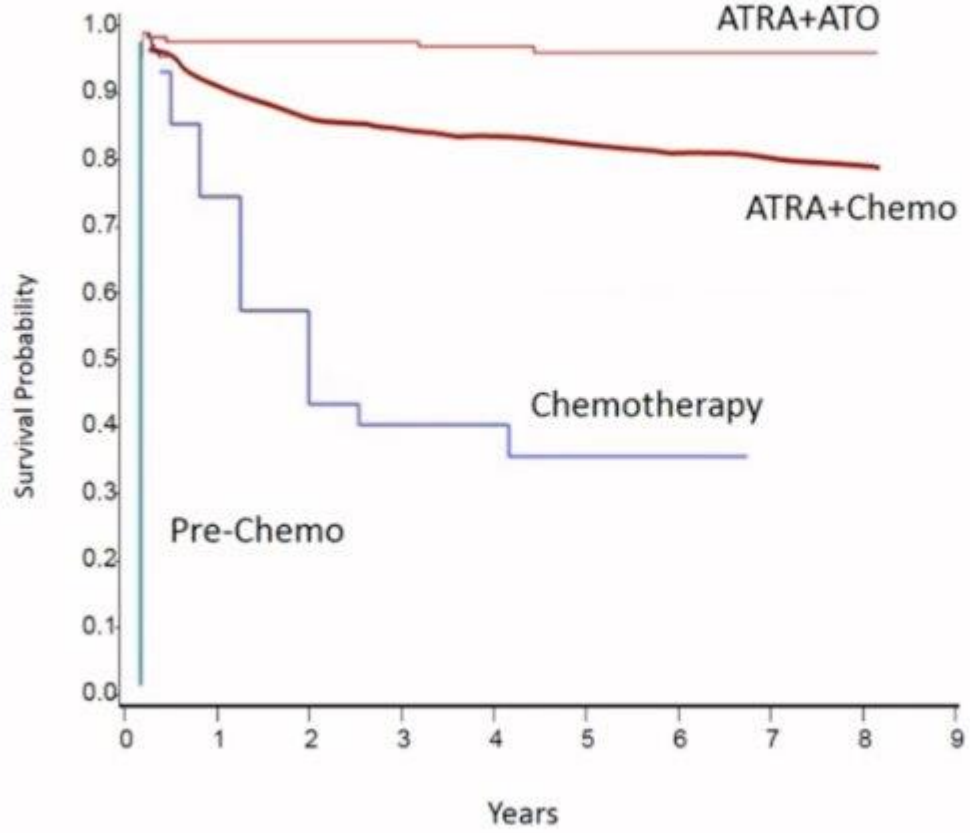
Risk Factors for Bleeding

- WCC >10 x10^9/L
- Fibrinogen <1.0 g/L
- PS <2
- High creatinine
- Platelets NOT predictive

SUPERHERO OR VILLAIN?



APL: From Highly Fatal to Highly Curable



ATRA: All Trans Retinoic Acid; ATO: Arsenic Trioxide

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UK NEQAS Haematology
Knizocytes … bless you

Andrea Teuchert

Morphology EQA Lead Scientist, UK NEQAS Haematology

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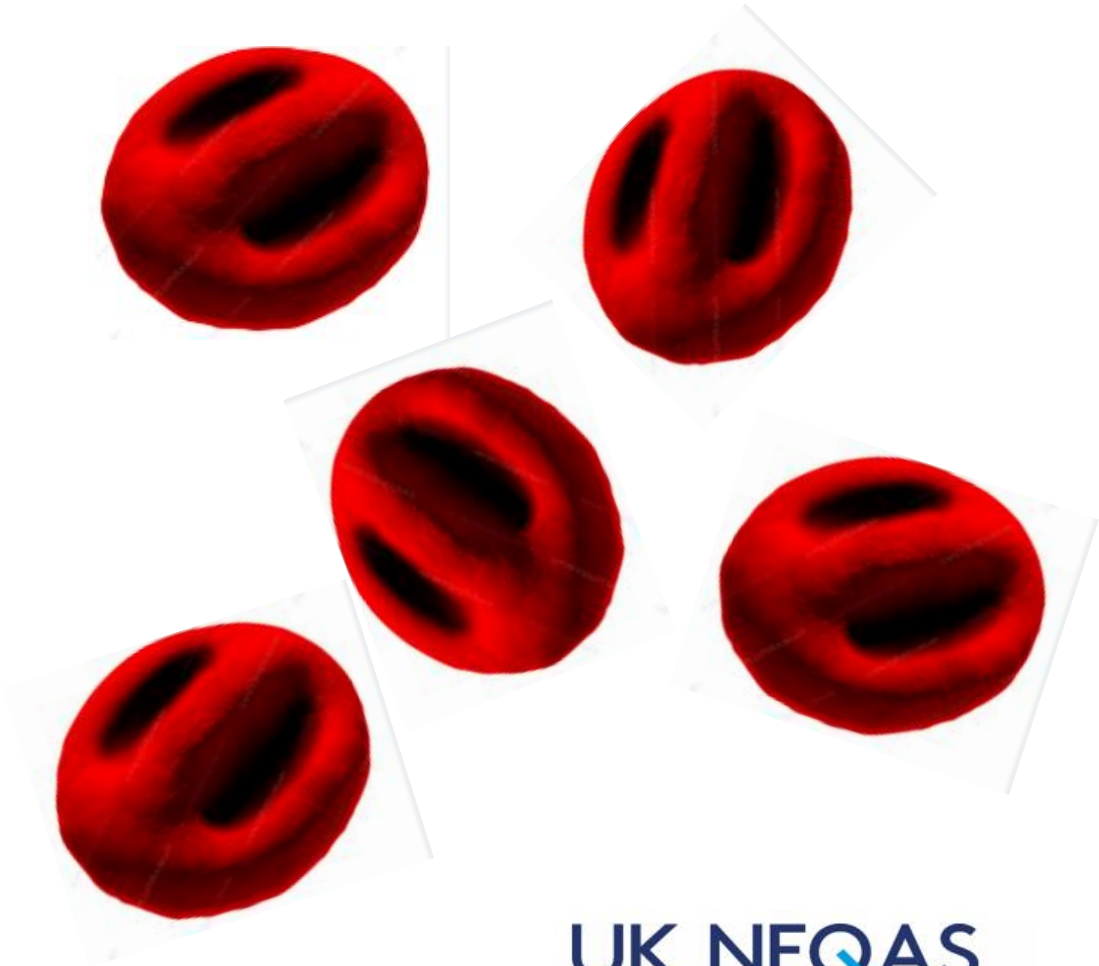
Red blood cells

- Red blood cells aka erythrocytes
- Most common type of cell in your blood
- Make up approximately 40 – 45% of its volume
- Primary role to transport oxygen throughout the body using a protein called haemoglobin
- Small, bi-concave cells produced in the bone marrow

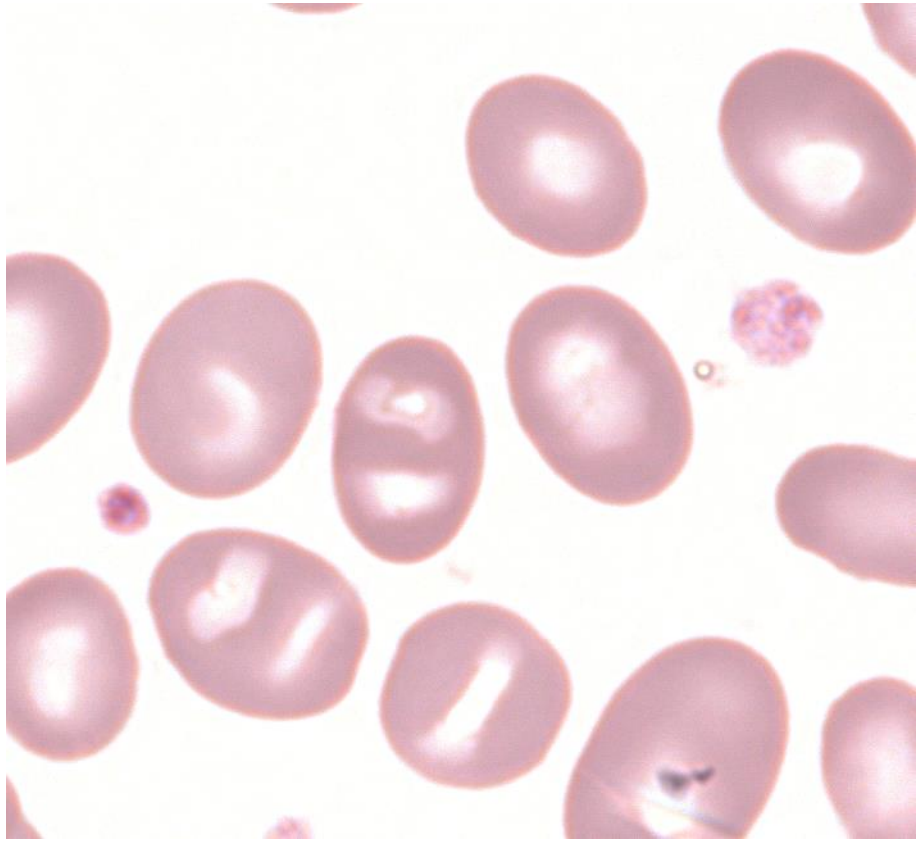
Knizocytes

- Eccentric red blood cells that have a ‘pinched’ look
- Triconcave with a ridge or bridge separating the concavitites
- Sometimes described as ‘double slit’ or ‘Y-shaped’ appearance
- Genetic conditions, chronic liver onditions and thalassaemia



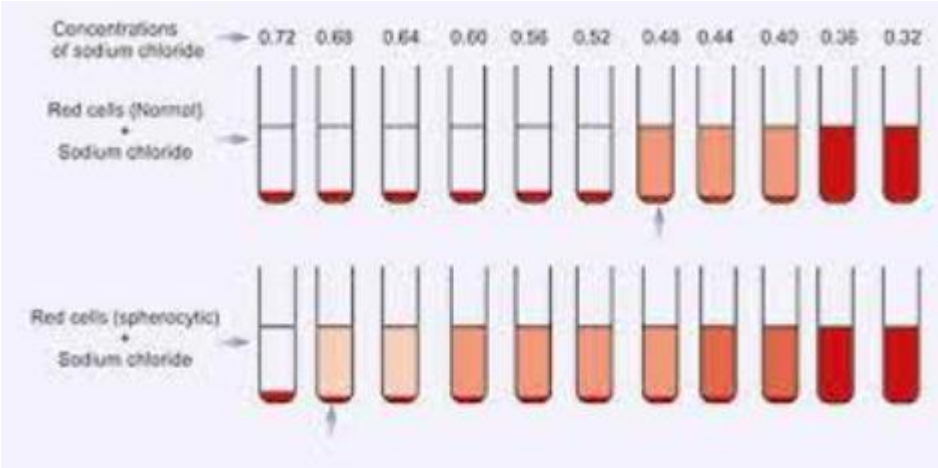
South East Asian Ovalocytosis

- Knizocytes are almost diagnostic
- Autosomal Dominant condition
- Usually asymptomatic
- Incidental discovery in adults
- Some patients experience mild haemolysis, anaemia and gallstones
- Distinct from hereditary elliptocytosis

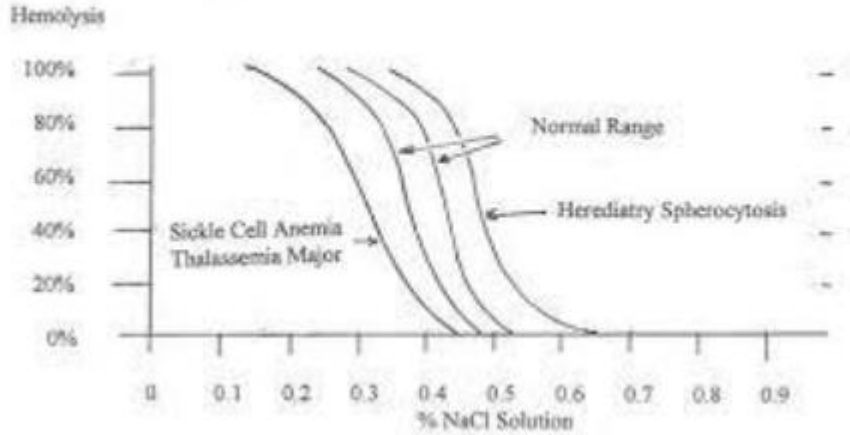


South East Asian Ovalocytosis cont.

- Band 3 protein in the red cell membrane
- Red cell membrane is more rigid
- More prone to osmotic fragility



Typical Graphs for RBC Osmotic Fragility



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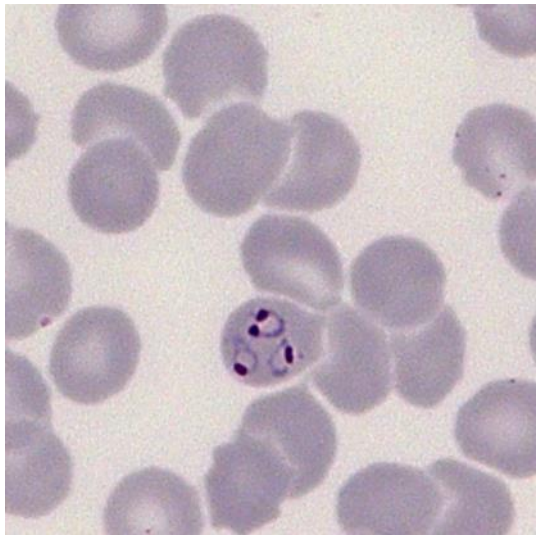
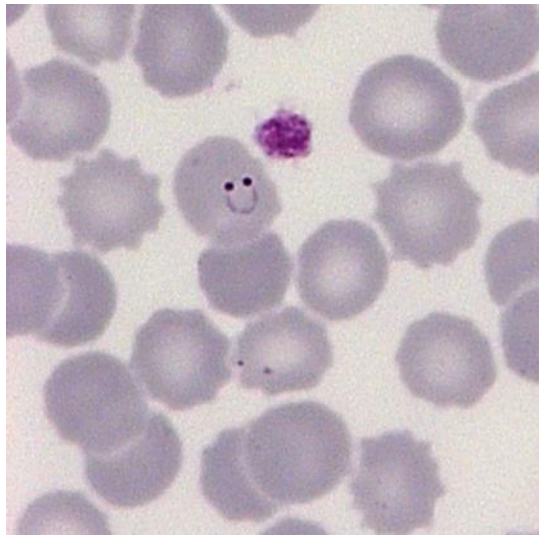
South East Asian Ovalocytosis cont.

- Protection against malaria infection
  - *Plasmodium falciparum*
  - *Plasmodium knowlesi*
  - *Plasmodium vivax*
- Natural selection





South East Asian Ovalocytosis cont.

- Common in populations of Island Southeast Asia, the Malay Peninsula, Papua New Guinea, the Philippines and Indonesia

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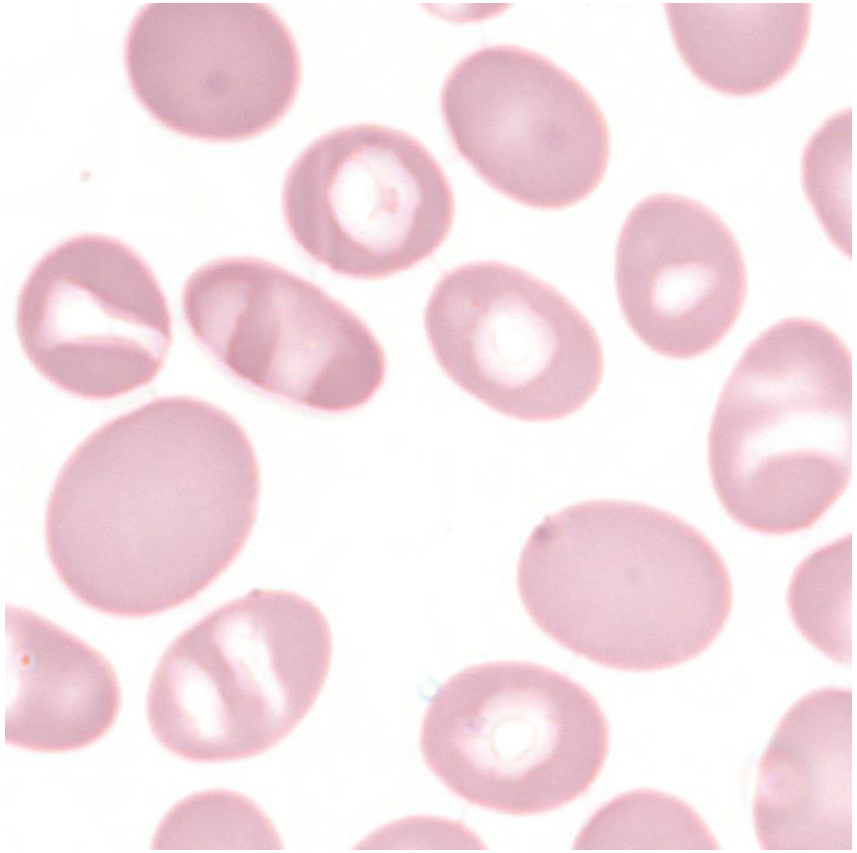
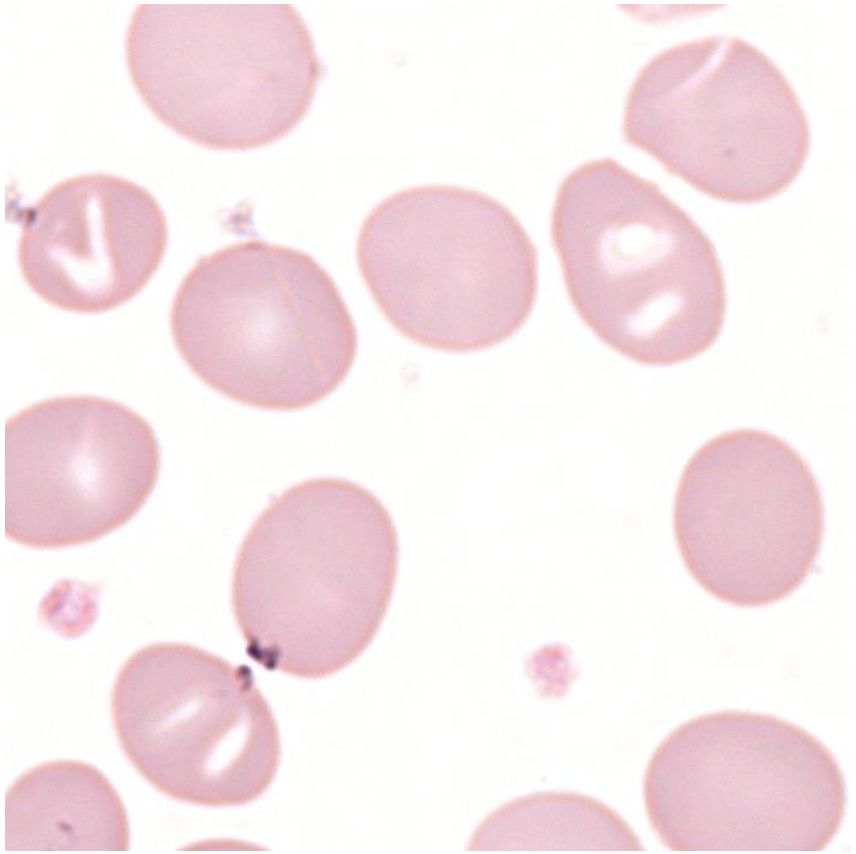
Favourite cell





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- If you have a sample or know of a case that we might be able to use, please call our department on **01923 587111** – please don't email, we may not pick up your email until it is too late. We will then arrange a courier to collect or one of our team can come and collect the sample from you – you don't need to do anything else except arrange for the sample to be packed securely.

- We ideally require:
  - The sample to be as fresh as possible, preferably less than 24 hours old and ideally available before 13:00 in order for it to get back to us in good time to make the slides we need
  - A full EDTA sample (we make 700+ slides so the more sample the better!)
  - We do not need patient consent if the sample has been taken for routine pathology testing and is 'spare'.
  - If you can include a copy of the FBC and preferably the chemistry, either with the sample or promptly via email, from the day the sample was taken, that is ideal. We can arrange to collect any Immunophenotyping, Bone Marrow report, genetics, etc at a later date
  - The sample and FBC reports etc. do need to be made anonymous, but please leave enough information for us to identify it with your lab at a later date. So please leave the patient's initials, DOB, and laboratory reference number.
  - Any information will be treated in complete confidence. All patient identifiable information is completely stripped from our final EQA reports before they are released.

Acknowledgements

- Thank you to the following labs for sending cases this past year:
  - Barts Health NHS Trust
  - North Midlands and Cheshire Pathology Service
  - North West London Pathology
  - South West London Pathology

- You are our morphology champions and without you we would not have so many great cases to send to all our participants

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