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TRIOLAB MSD Animal Health

Fredag 15. mars

Program for Smådyr

The emergency patient – examination of blood smears

the most common erythrocyte and leukocyte morphological abnormalities & their clinical significance

Kostas Papasouliotis

DVM PhD DipECVCP MRCVS

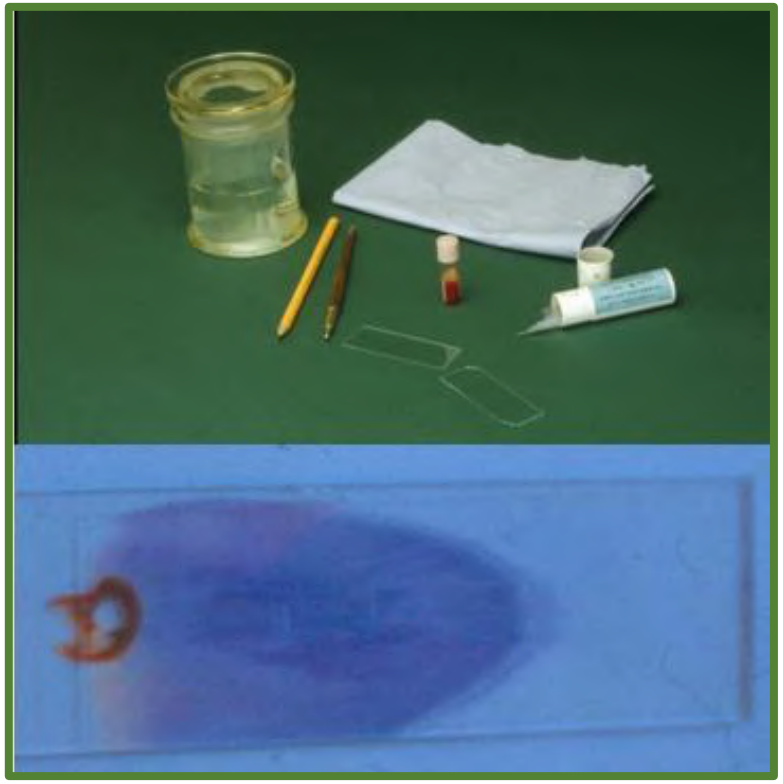
EBVS® European Specialist in Veterinary Clinical Pathology

Diagnostic Laboratories, Langford Vets, Bristol Veterinary School, University of Bristol

kos.papasouliotis@icloud.com

Introduction

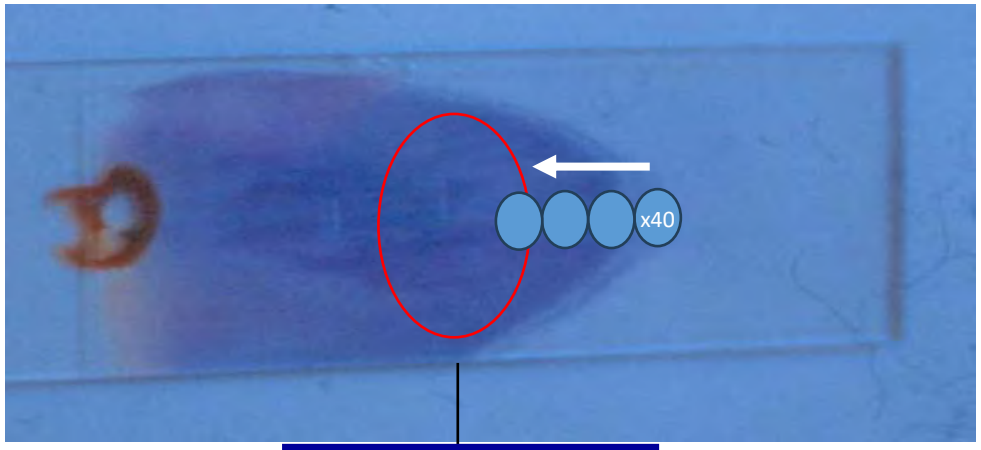
- Accurate laboratory testing is vital.
- Clinical pathology services not available immediately and/or after hours.
- In-clinic haematology analysers are essential BUT do not provide complete haematological assessment.
- Blood smear microscopic examination is essential.



BLOOD SMEAR

x40

x100



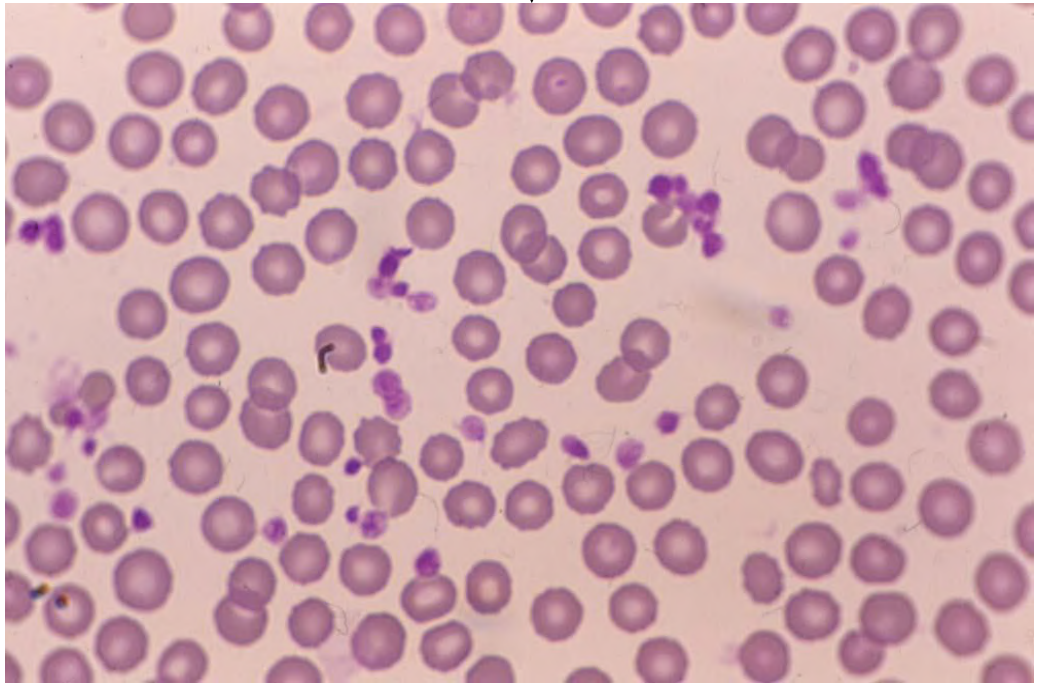
Monolayer

PRACTICE – PRACTICE- PRACTICE

-Plan your day so you can have time for Blood smear examination

-Comfortable area

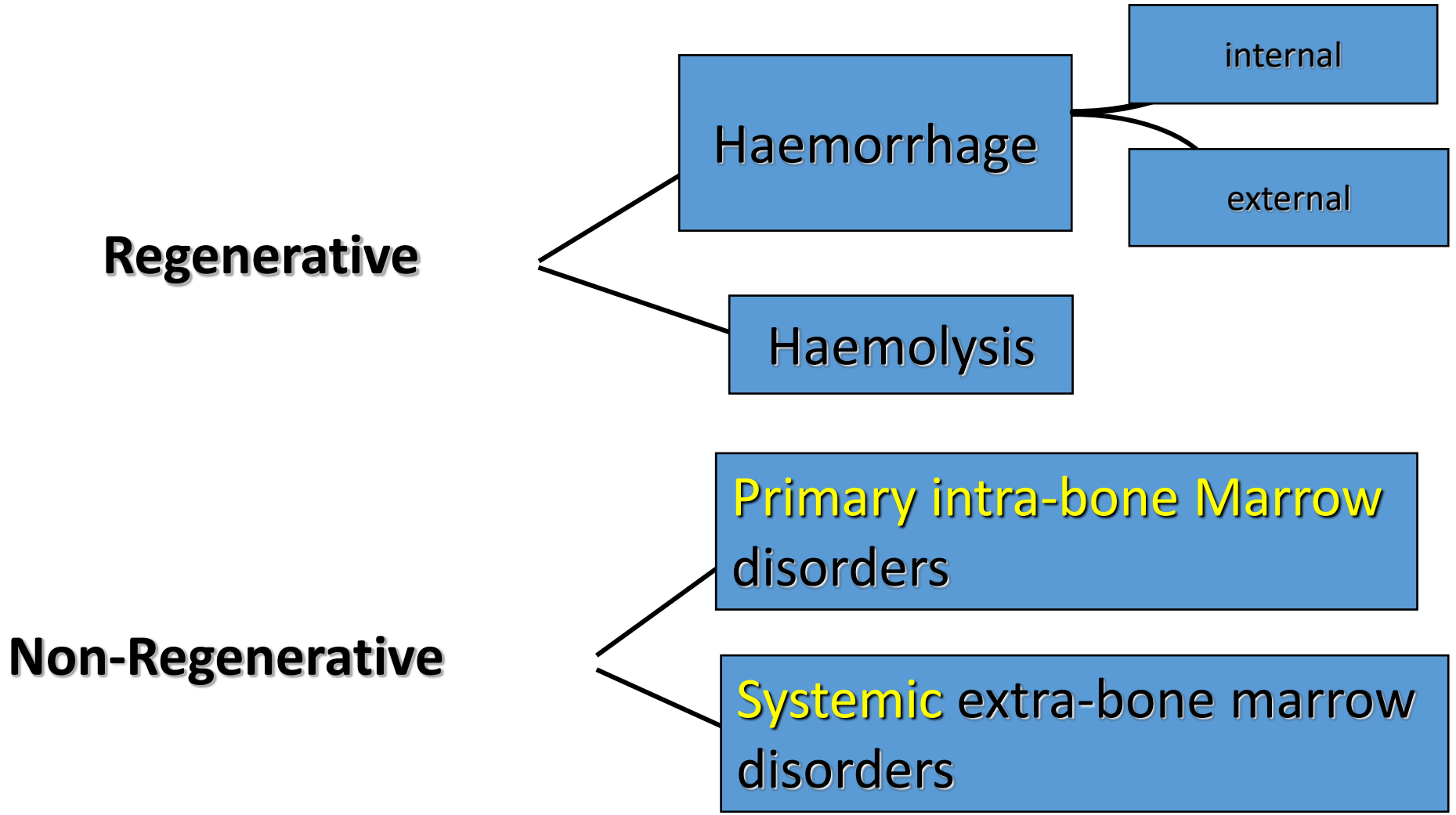
Invest in a good microscope - Take care of your instrument



Blood smear – the emergency patient

- Is the anaemia regenerative?
  - Essential when the Haematology analyser does not provide a Reticulocyte count
- Is the anaemia an IMHA?
- Is there evidence of active overwhelming inflammation?
- Is a Neoplastic process present?
  - Presence of atypical/abnormal cells
- Is the patient Thrombocytopenic?
- Any parasites present?

Anaemia: DECISION MAKING

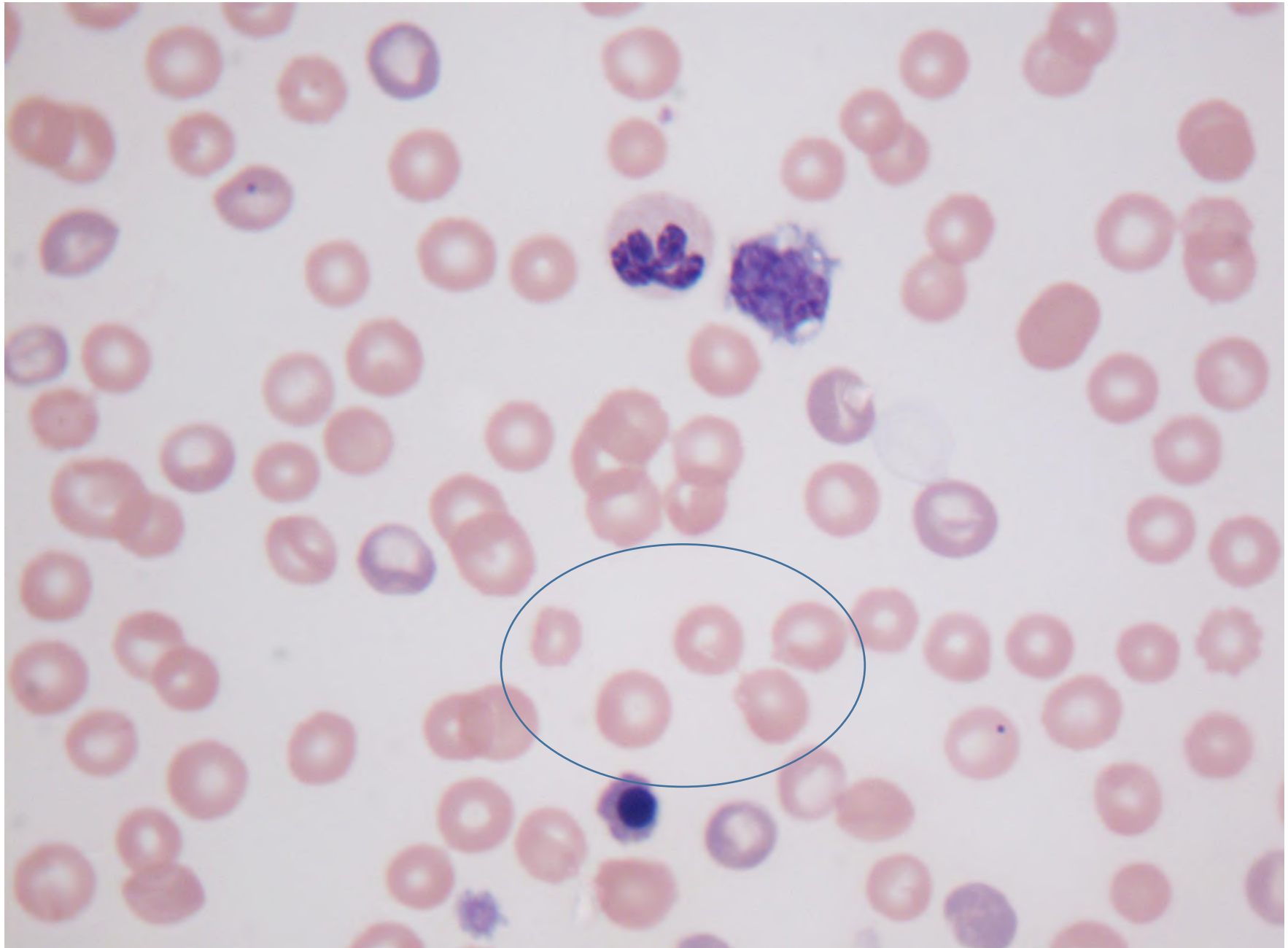


The emergency patient – Regenerative vs Non-regenerative anaemia

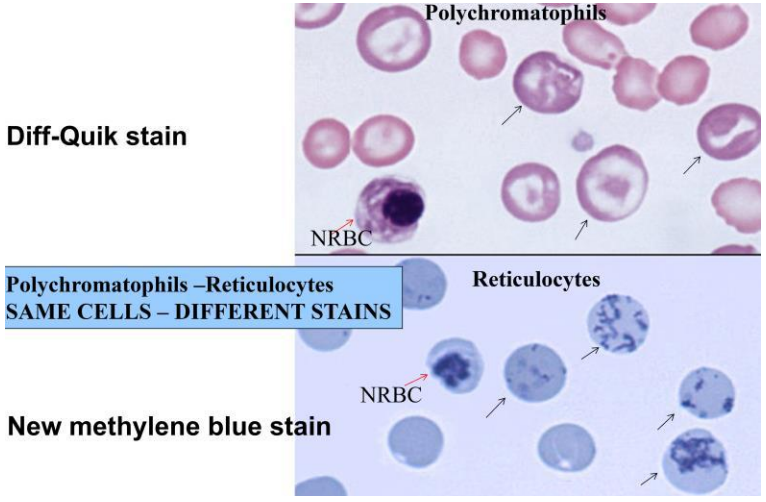
The anaemia is REGENERATIVE when there is:

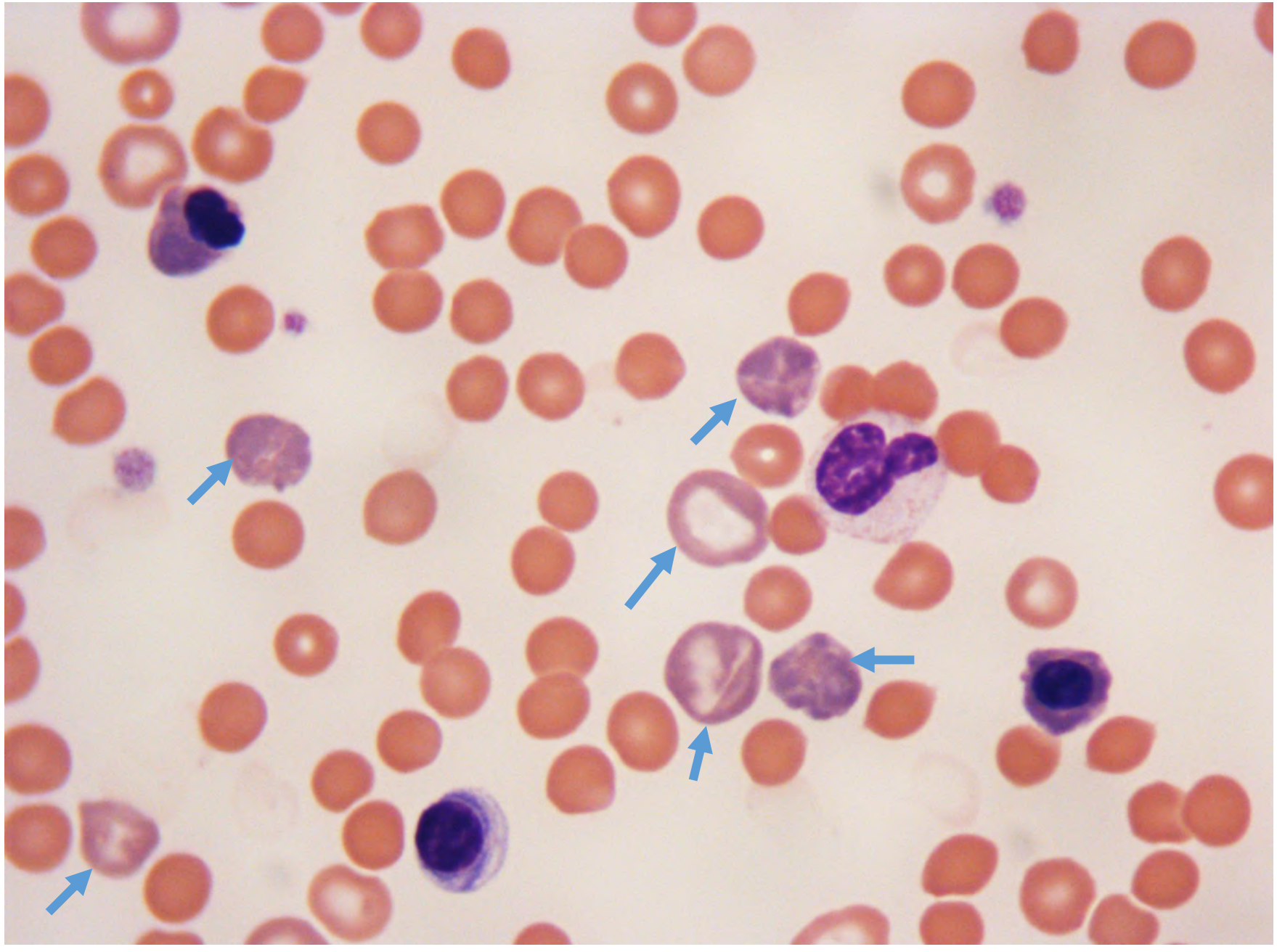
- Anisocytosis = RBCs of different sizes
- Polychromasia = Presence of Polychromatophils (=Reticulocytes)
- Presence of Nucleated red blood cells (nRBC)

Anisocytosis = RBCs of different sizes



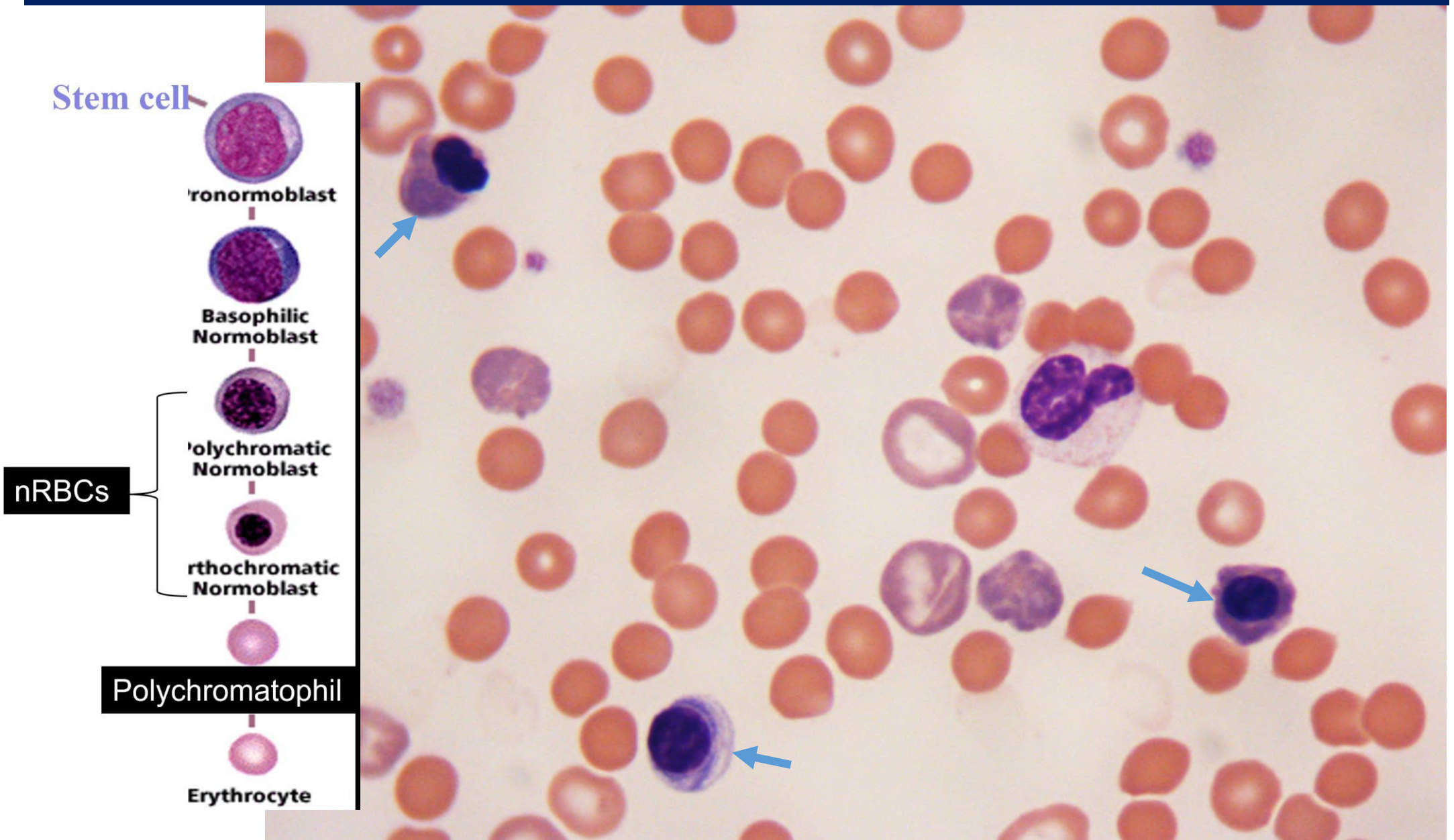
Polychromasia = Presence of polychromatophils



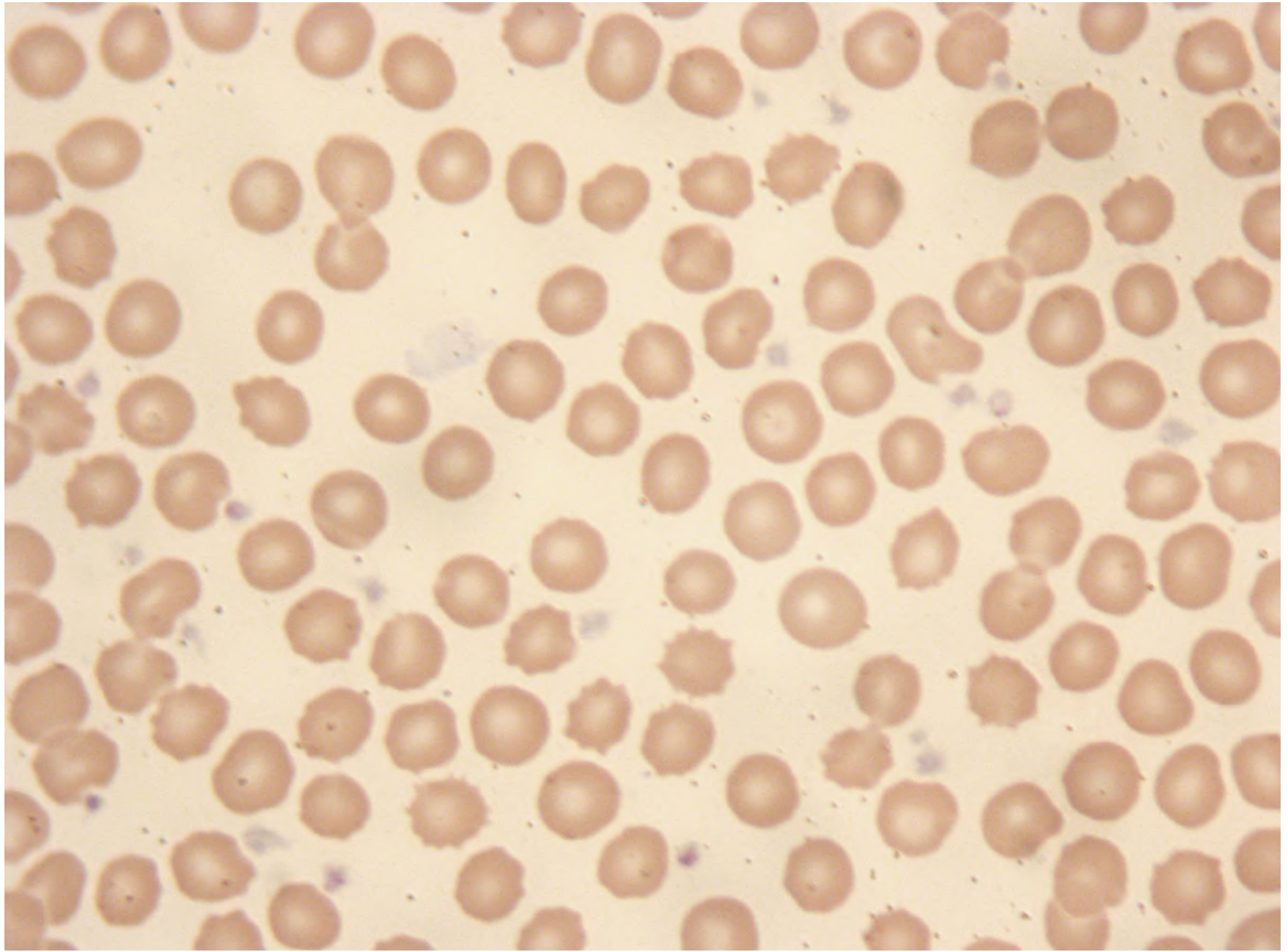


Presence of Nucleated red blood cells (nRBCs)

Immature erythrocytes = “younger” than polychromatophils (reticulocytes)



Anisocytosis, Polychromasia, nRBCs are not present = Anaemia is non-regenerative



The erythrocytes are
normocytic
and
normochromic

Blood smear – the emergency patient

- Is the anaemia regenerative?
  - Essential when the Haematology analyser does not provide a Reticulocyte count
- **Is the anaemia an IMHA?**
- Is there evidence of active overwhelming inflammation?
- Is a Neoplastic process present?
  - Presence of atypical/abnormal cells
- Is the patient Thrombocytopenic?
- Any parasites present?

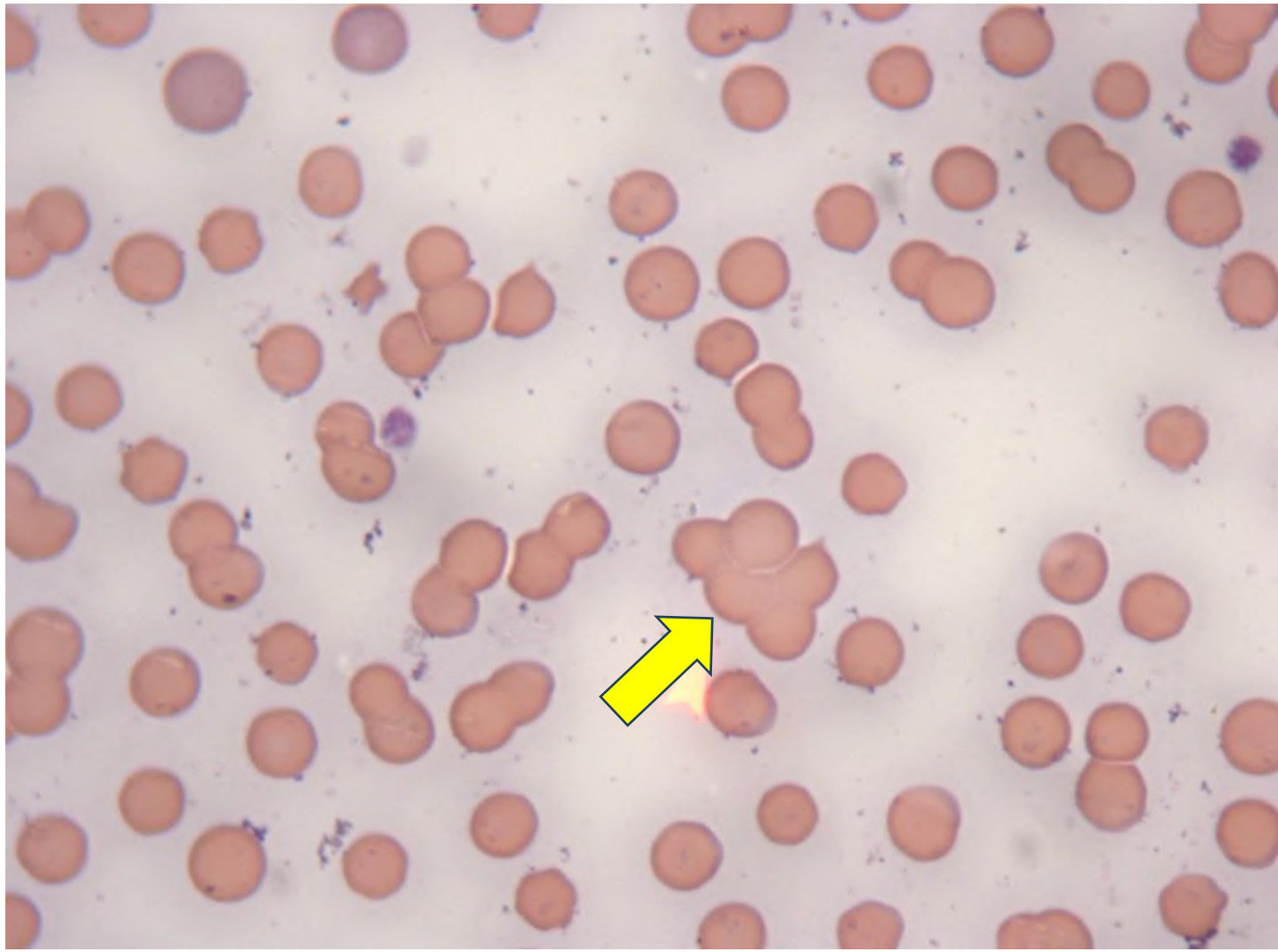
The emergency patient – IMHA

- Agglutination
- Spherocytes
- Ghost cells

Immune-mediated haemolytic anaemia

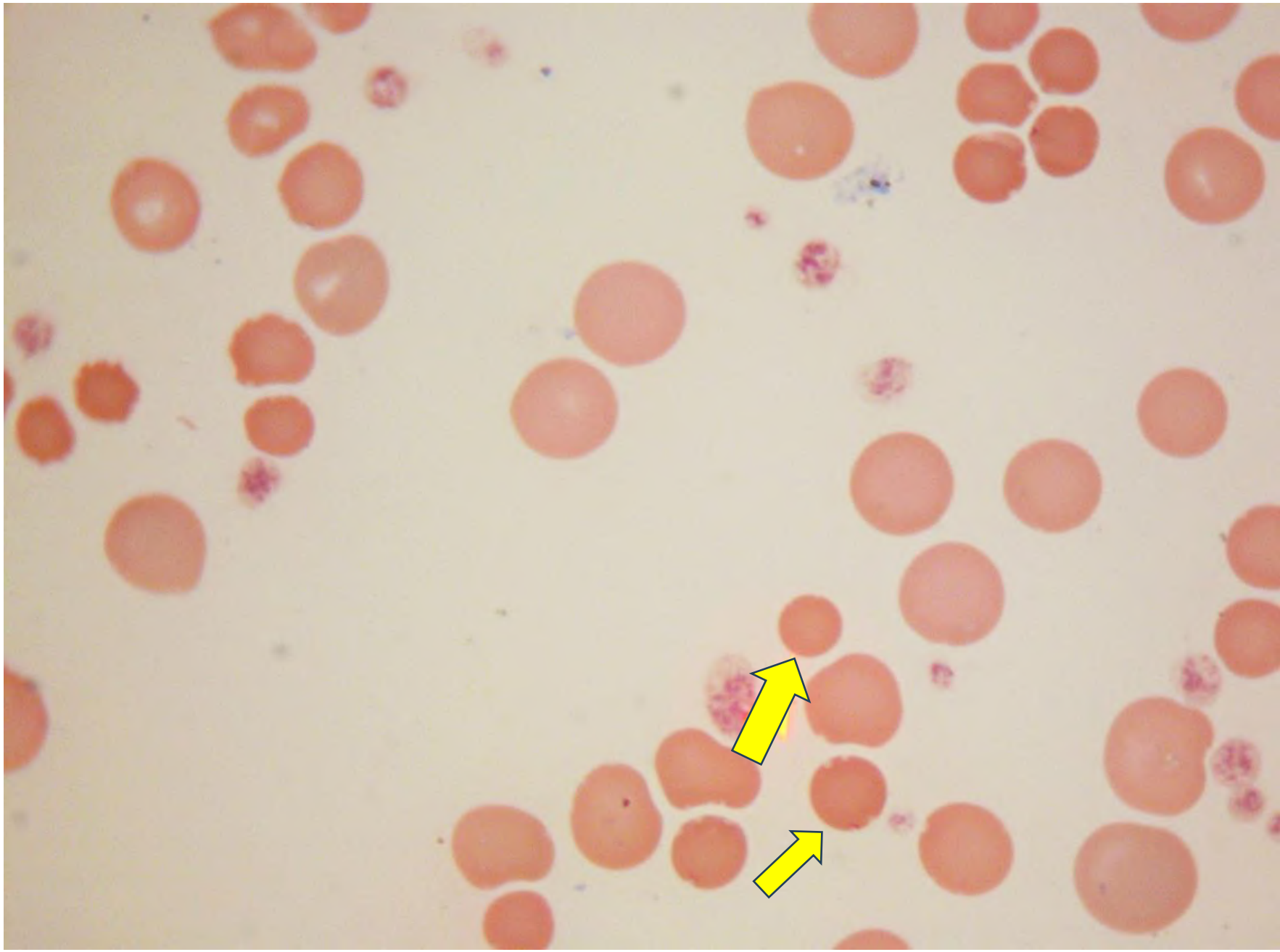
RBC agglutination

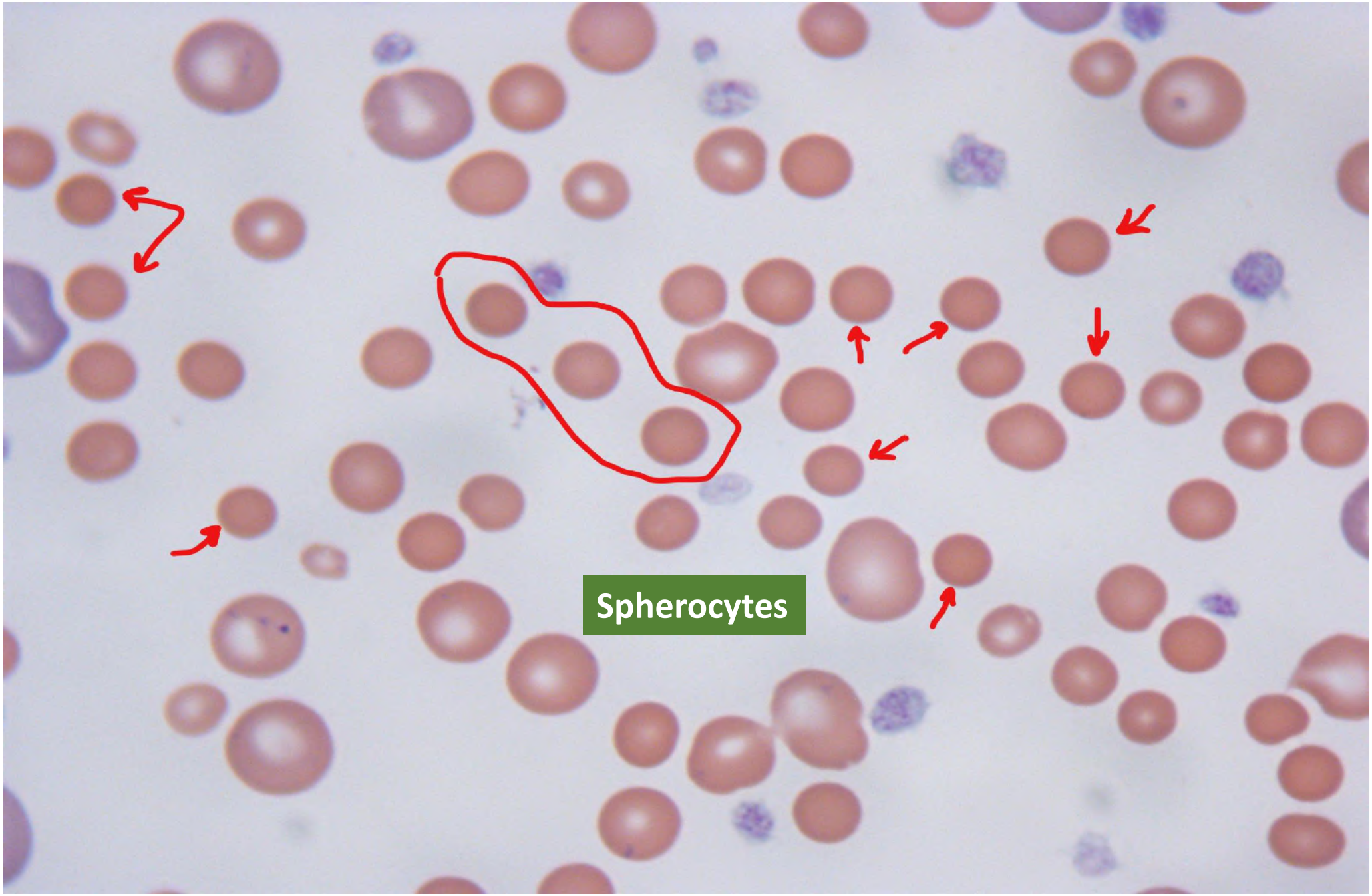
- Unorganized 3-dimensional clustering of RBCs
  - cross-linking of RBC surface Ab
- Distinguish from rouleaux (**R**)
  - Electrical charges on RBCs

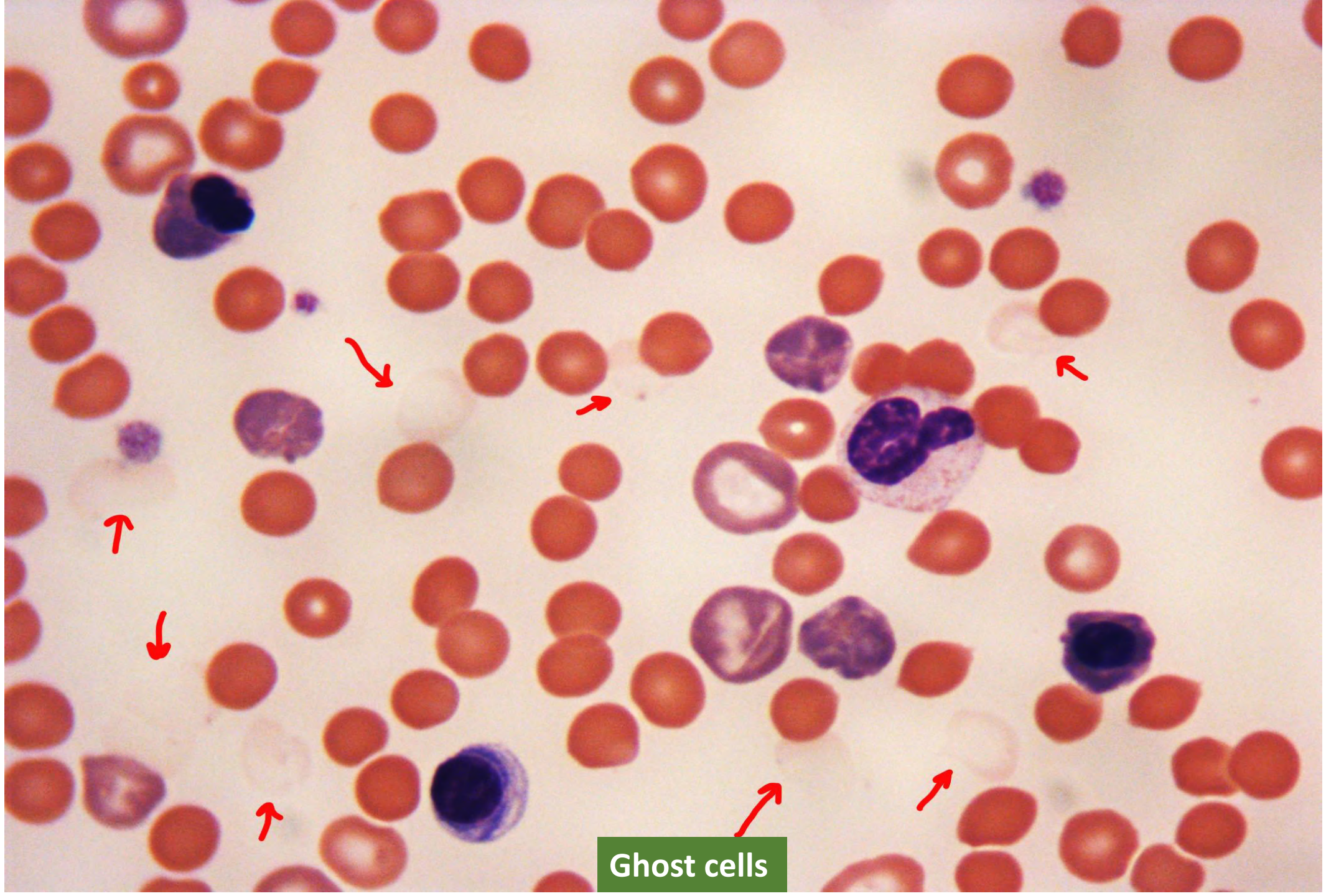


Spherocytes

- Formed when
  - Macrophages partially remove Ab coated RBC membranes
  - Membrane loss
  - spherical shape
- Difficult to recognize spherocytes in cats
  - (normal feline erythrocytes doe not exhibit central pallor)




Spherocytes


Ghost cells

Blood smear – the emergency patient

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- Is the anaemia an IMHA?
- **Is there evidence of active overwhelming inflammation?**
- Is a Neoplastic process present?
  - Presence of atypical/abnormal cells
- Is the patient Thrombocytopenic?
- Any parasites present?

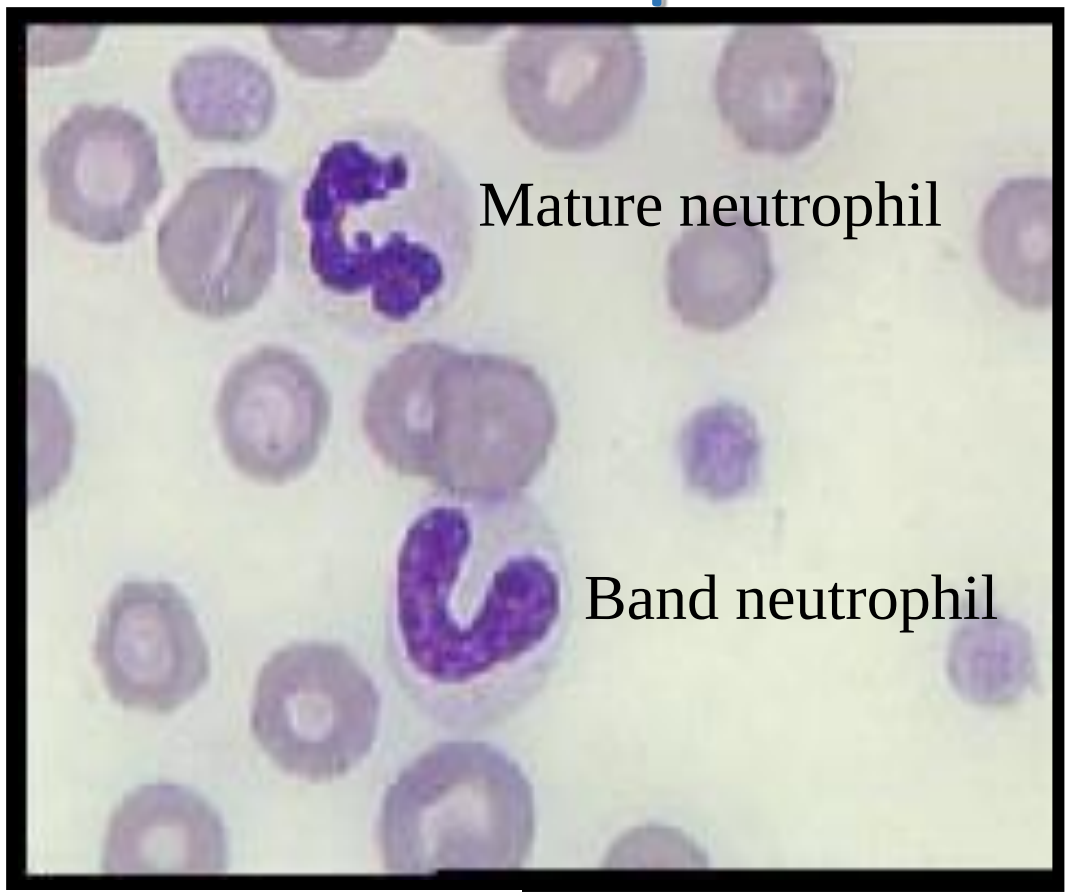
- **Immature neutrophils**
- **Toxic neutrophils**

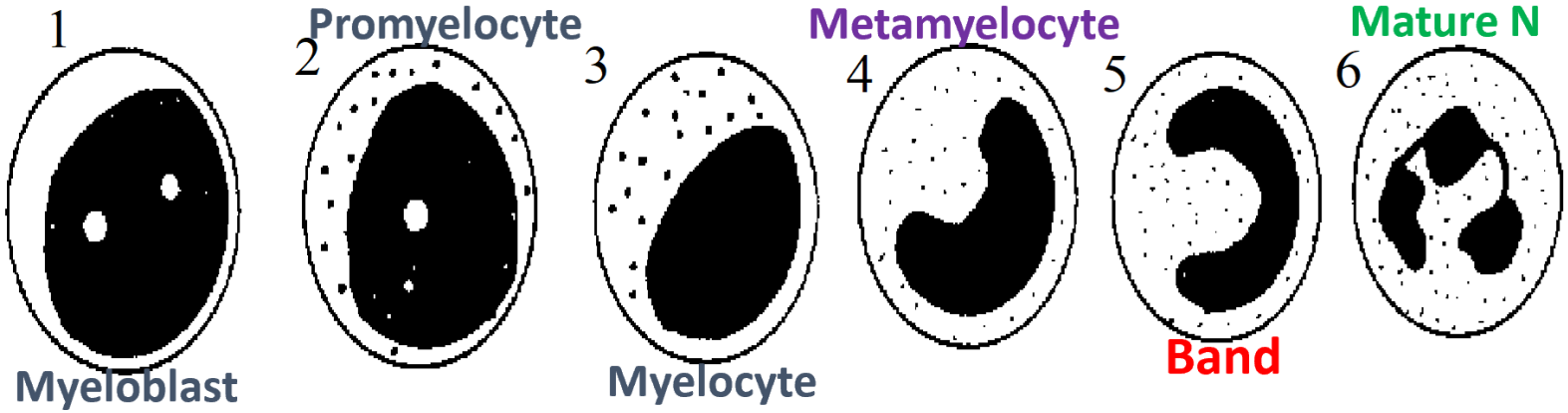
Active inflammation/infection
Overwhelming inflammation/infection

The emergency patient – Inflammation

Immature neutrophils -1

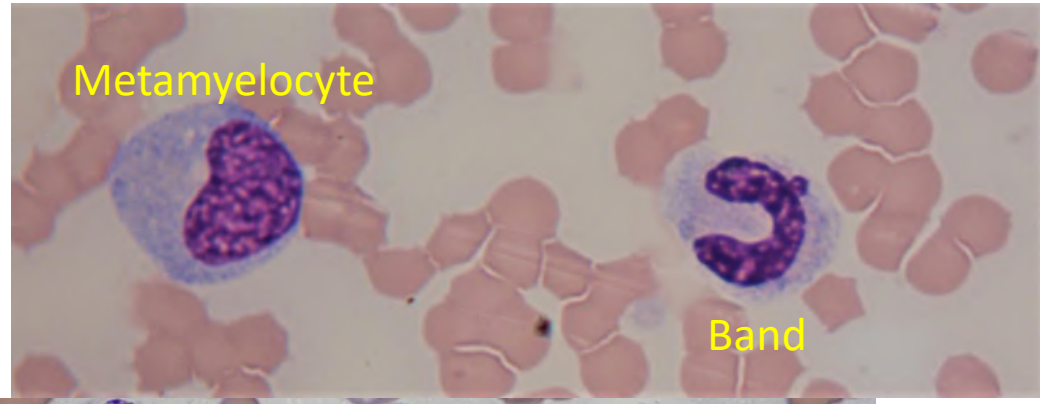
- Left shift (presence of immature neutrophils)
  - commonly
    - Band N ±Metamyelocytes
    - > 1 cell per 10 fields (x40 lens)
- Two types of left shift:
  - Regenerative
    - Left shift + Neutrophilia
  - Degenerative
    - More immature than mature neutrophils

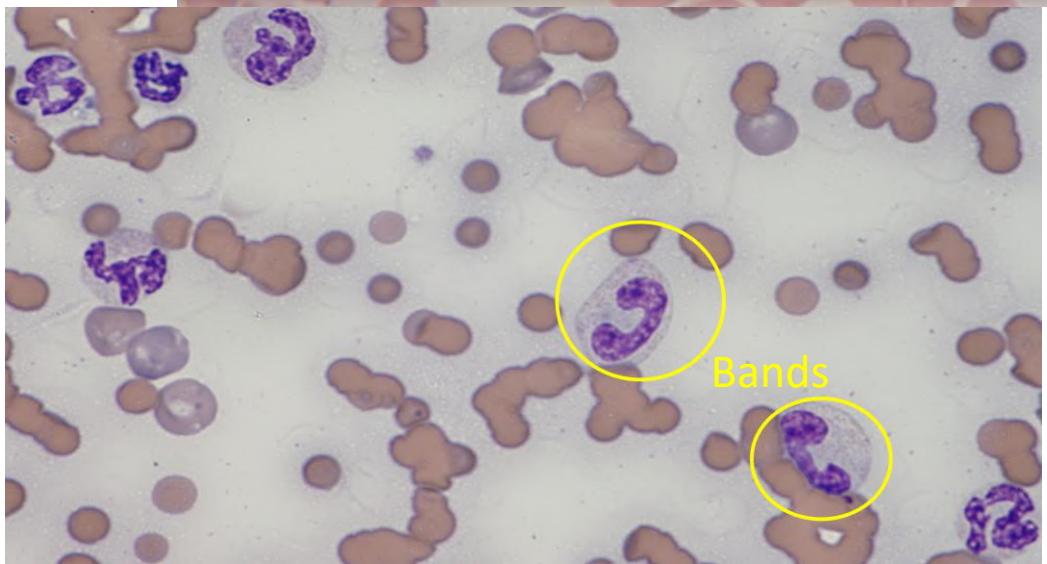




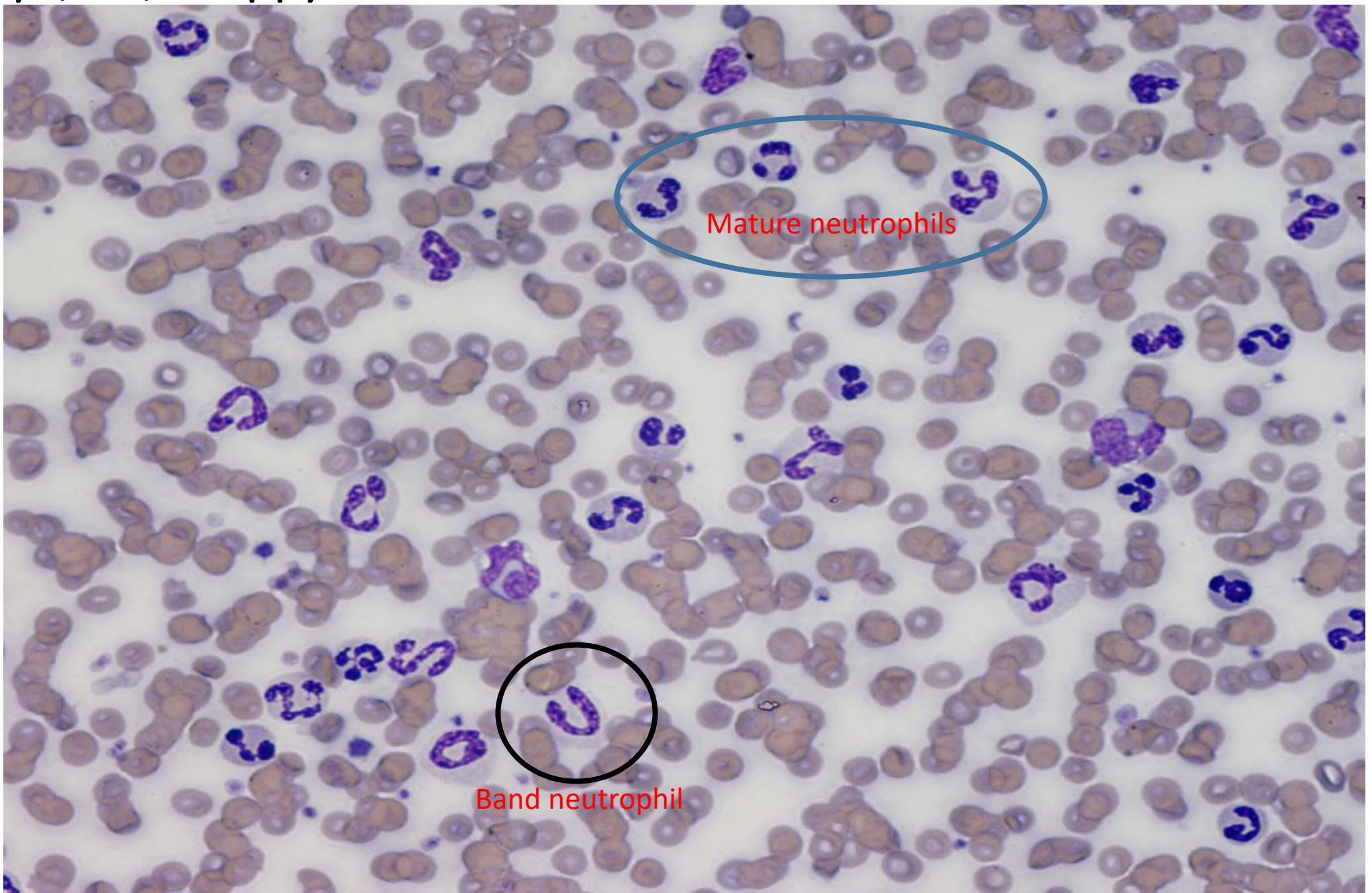
Immature neutrophils -2

- **Neutrophilia with left shift**
  - Inflammatory demand
  - Depletion of the Neuts storage pool in the bone marrow
  - Less common in cats
- **Degenerative left shift (immature N > mature N)**
  - Rate neutrophil destruction higher than production in BM
  - BM cannot keep up with demand
  - Overwhelming inflammation
- **Causes for the presence of immature N**
  - Purulent inflammation
  - Intense inflammation
    - Infection
    - Extensive cell damage
      - e.g. neoplasia,
      - immune-mediated dz



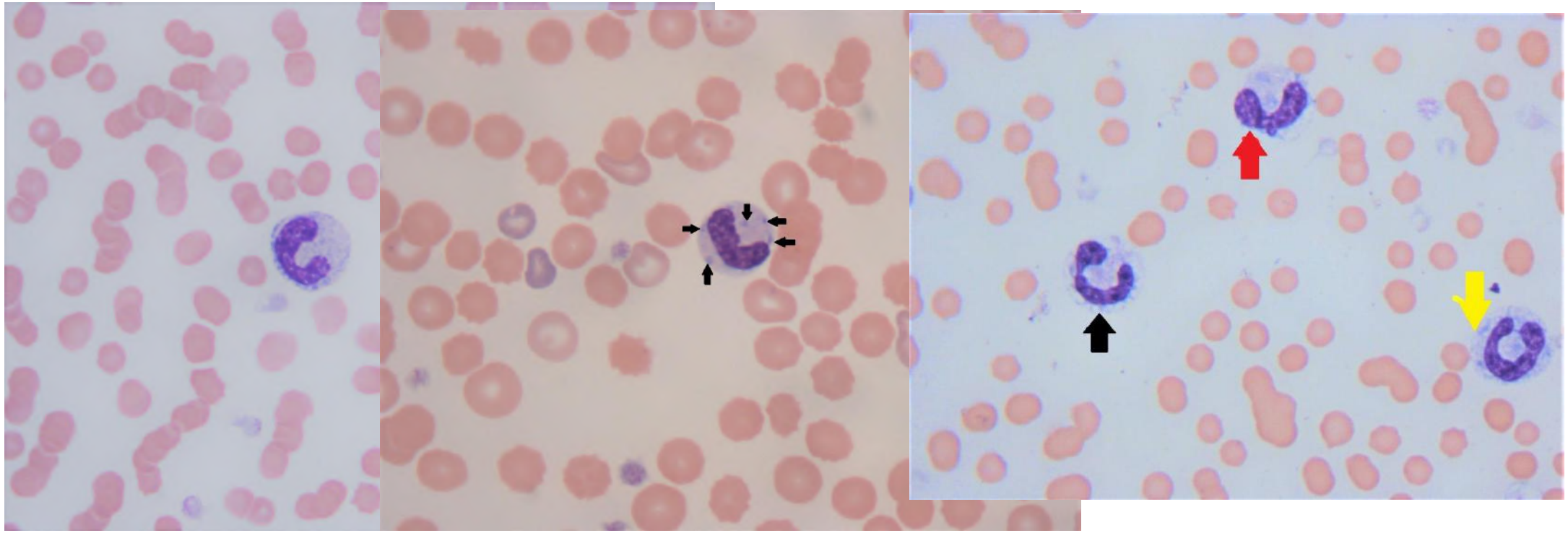


WHT, 5yo, FN, “Poppy”



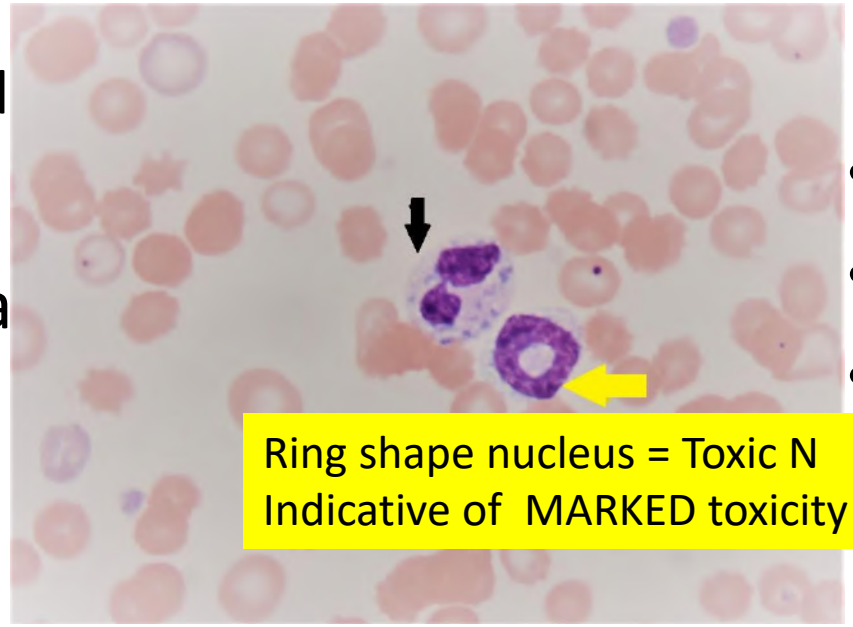
Toxic neutrophils – (can be mature or immature)

- Morphologically abnormal; develop in the Bone Marrow
  - Maturation asynchrony between nucleus and cytoplasm
  - Due to accelerated neutropoiesis (shortened maturation time) driven by cytokines in response to intense inflammation
- Cytoplasmic basophilia (increased amount of ribosomal RNA)
- Cytoplasmic foaminess (prominent lysosomes)
- Döhle bodies (bluish, irregular aggregates of rough endoplasmic reticulum)



Toxic neutrophils– Clinical significance

- **CATS**
- ~50% of cases with toxic neutrophils have normal WBC and Neuts counts
- Commonly seen with
  - Pneumonia, Upper respiratory tract infection
  - Parvovirus infection, FIV infection
  - Sepsis, Shock
  - Diabetic ketoacid lipidosis
- Longer hospitalisa
- Higher Tx cost



- **DOGS**
- Commonly seen with
  - Pyometra, Pancreatitis, Peritonitis
  - IMHA
  - Parvovirus infection
  - Sepsis, DIC
  - Neoplasia
  - Acute renal failure
- Longer hospitalisation
- Higher Tx cost
- Higher case fatality

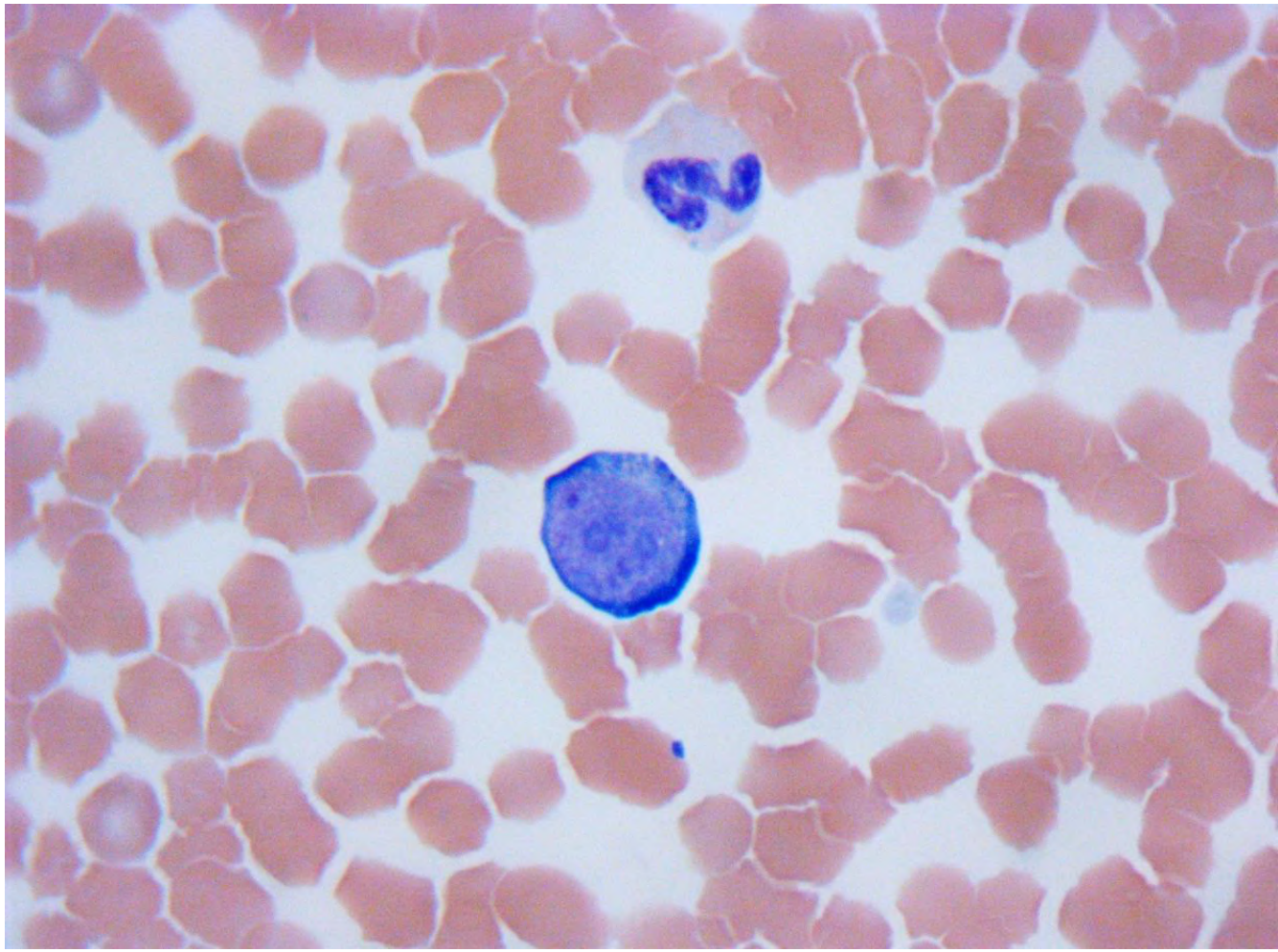
Blood smear – the emergency patient

- Is the anaemia regenerative?
  - Essential when the Haematology analyser does not provide a Reticulocyte count
- Is the anaemia an IMHA?
- Is there evidence of active overwhelming inflammation?
- **Is a Neoplastic process present?**
  - **Presence of atypical/abnormal cells**
- Is the patient Thrombocytopenic?
- Any parasites present?

The emergency patient – Neoplasia

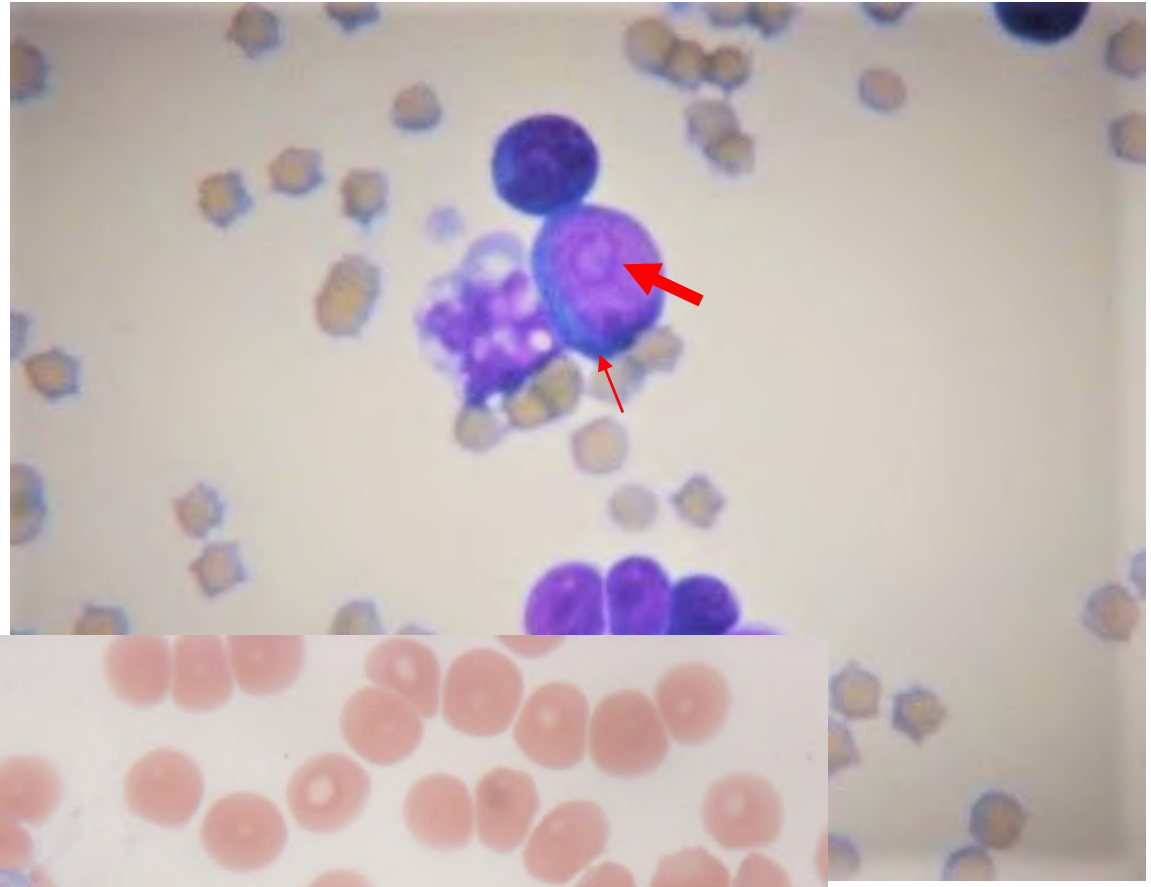
- Indicate a malignant neoplastic process (e.g. leukaemia).
- The lineage and origin of the cell is not always apparent.

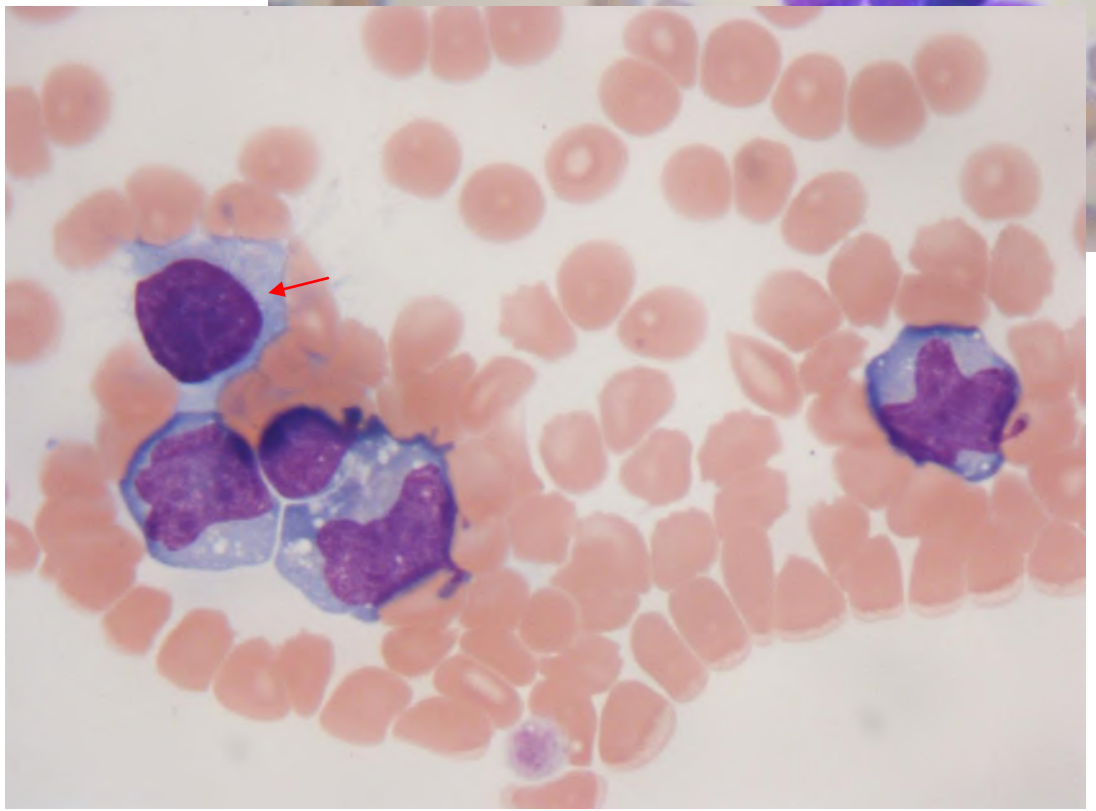
- Not easy to identify especially in an emergency case
- Even so, their detection
  - will identify the need for a clinical pathologist's opinion
  - significantly speed-up further diagnostic work and treatment



Blasts – Unclassified cells

- Have a single round, oval, indented, or convoluted nucleus.
- One or more prominent or indistinct nucleoli
- Variable amounts of lightly to markedly basophilic cytoplasm
- These cells may be lymphoid or myeloid in origin
  - Additional investigation by a clinical pathologist is needed
  - Special stains – Flow cytometry – Bone marrow biopsy & aspirate
- Clinical significance
  - Leukaemia (neoplasia of the bone marrow)





Blood smear – the emergency patient

- Is the anaemia regenerative?
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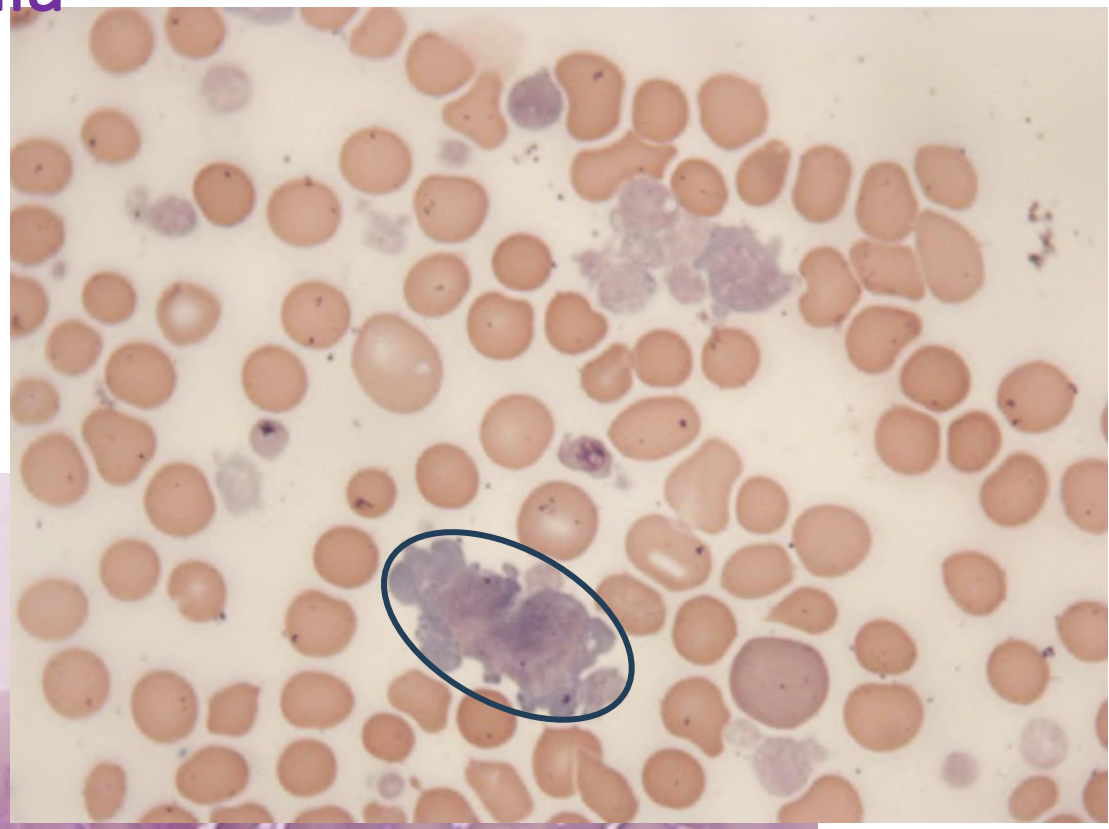
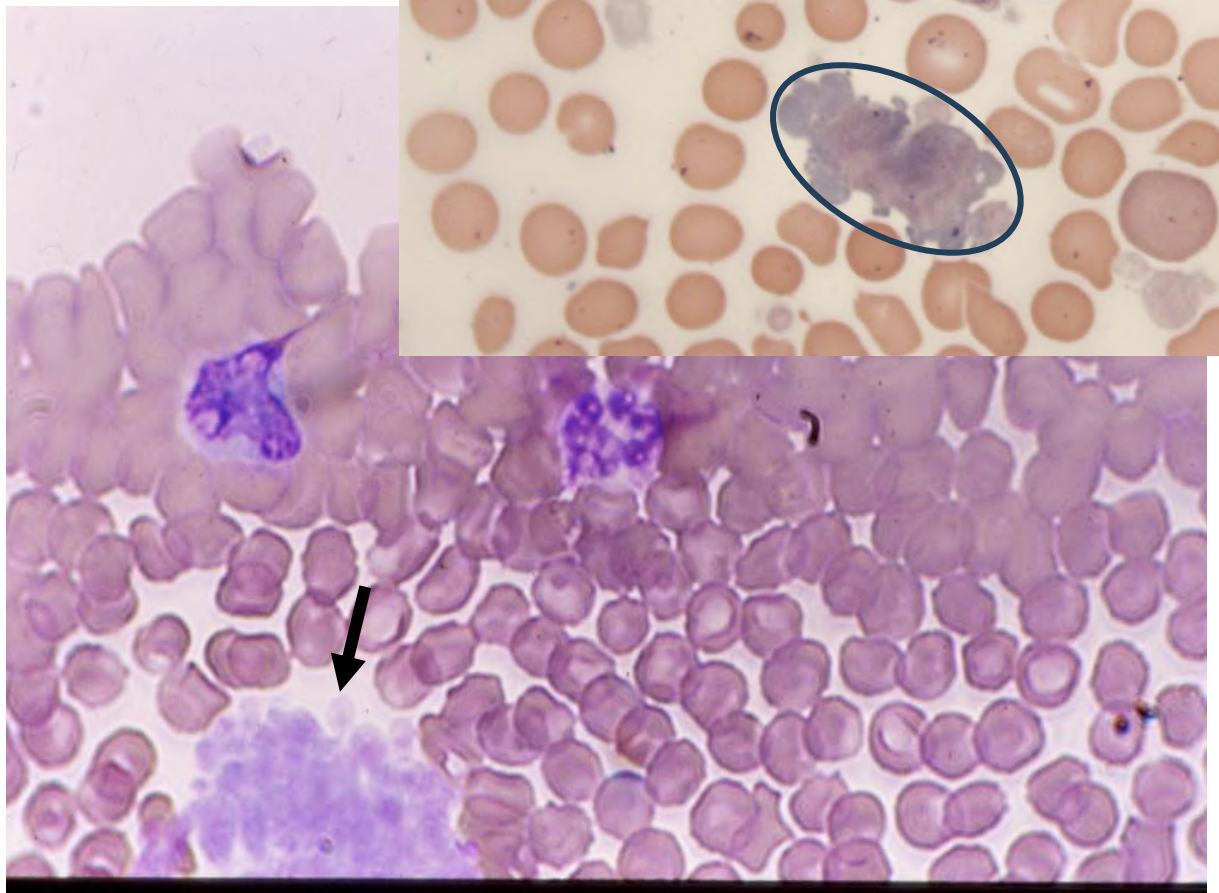
- Is the anaemia an IMHA?
- Is there evidence of active overwhelming inflammation?
- Is a Neoplastic process present?
  - Presence of atypical/abnormal cells
- **Is the patient Thrombocytopenic?**
- Any parasites present?

The emergency patient – Thrombocytopenia

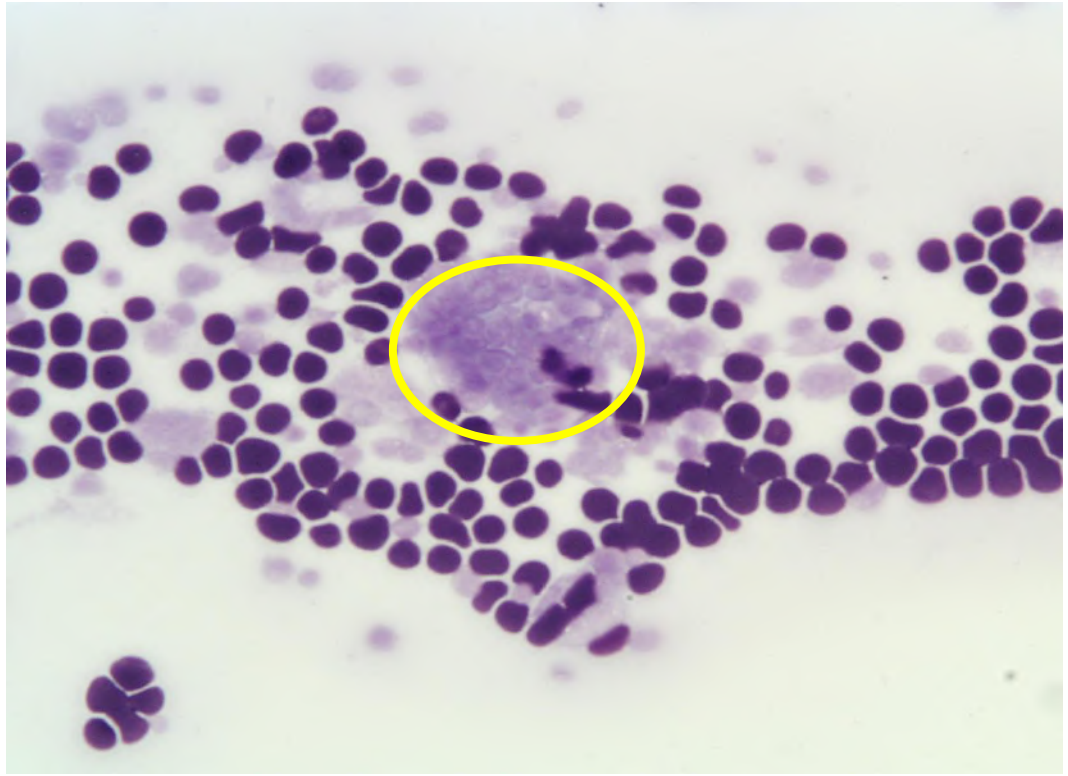
- Automated platelet counts from the haematology analysers
  - “false” thrombocytopenia
  - due to platelet clumping
  - Large platelets (macroplatelets)





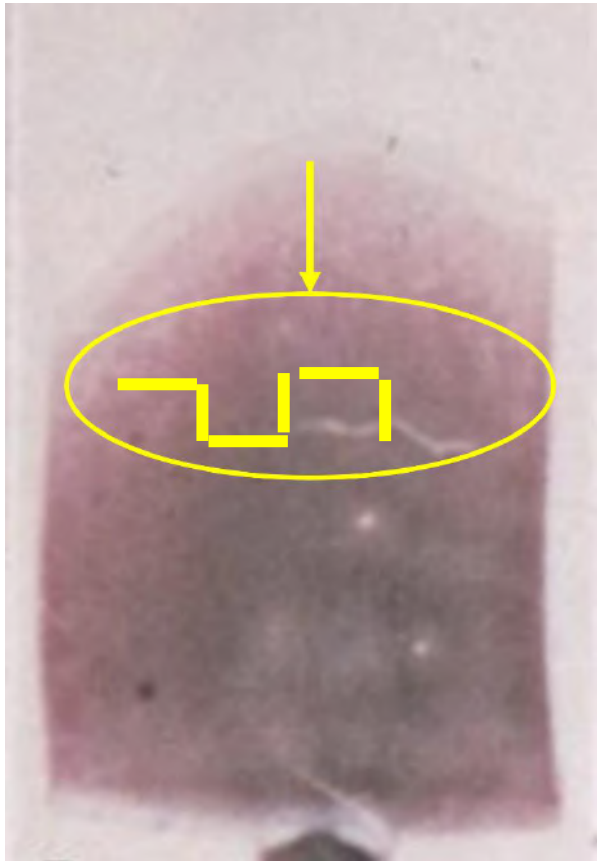
Manual platelet count estimation: Step 1

- Examine the feathered edge on x20 lens
  - Platelet clumps ? (if present, likely false thrombocytopenia)



Step 2

- MONOLAYER
- x1000 magnification (x100 oil lens)
- Count platelets in 10-20 fields
- Calculate the mean number of platelets



CAT: Multiply Mean number x 20 = Total number ($x10^9/L$)

DOG: Multiply Mean number x 15 = Total number ($x10^9/L$)

Causes - Thrombocytopenia

- Decreased production
  - Bone marrow dz
  - Drugs,FeLV/FIV
- Loss/Sequestration
  - Blood loss
  - Sequestration in spleen
- **Increased utilization:**
  - DIC, vasculitis
- **Destruction:**
  - Immune mediated

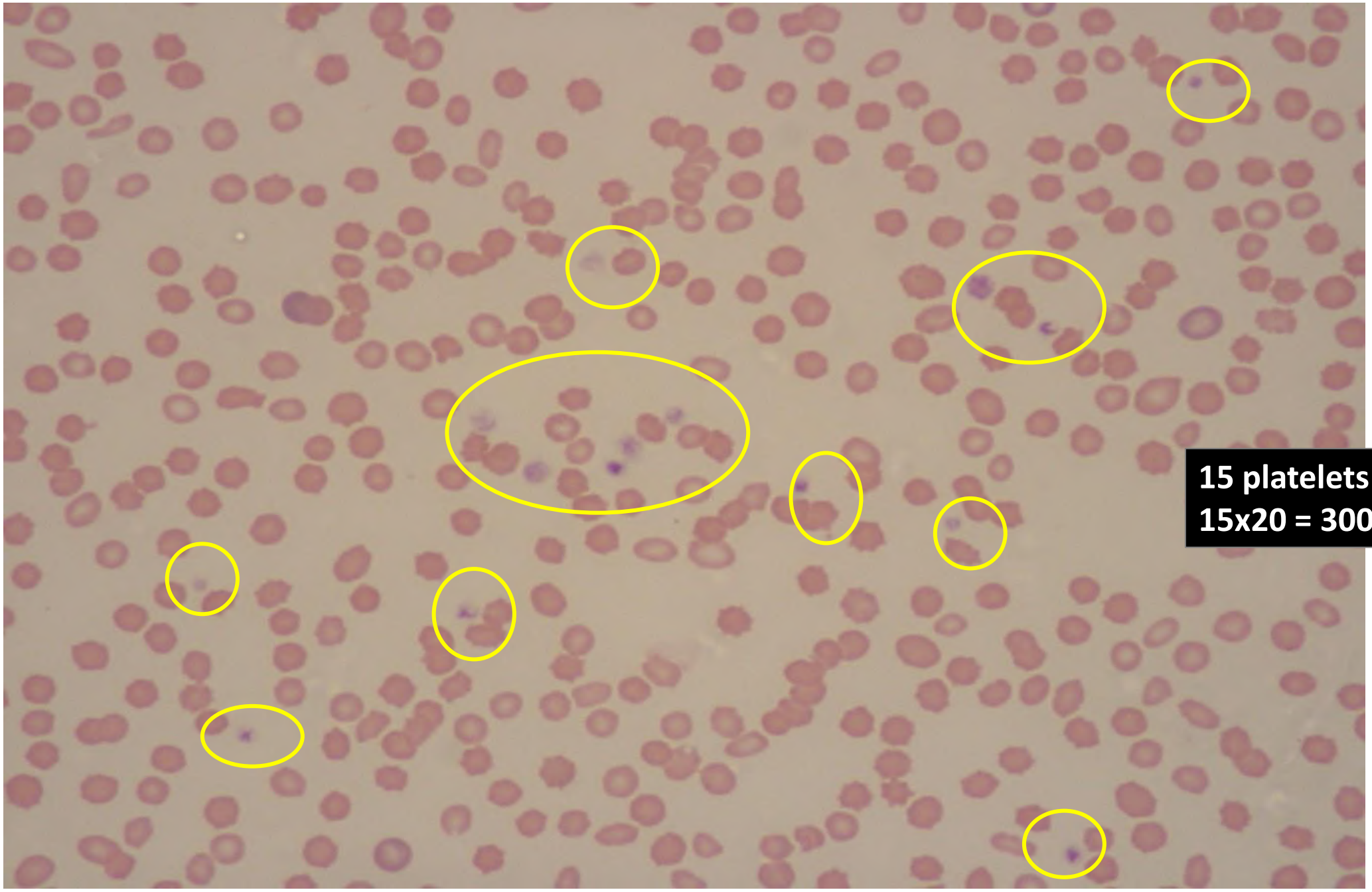
Most common causes

HAEMATOLOGY Cat

Test	Result	Alert	Units	Reference Range
Red cells	7.27		10^12/L	7.12 - 11.46
Haemoglobin	10.4		g/dL	10.3 - 16.2
Hct	0.344		l/L	0.282 - 0.527
MCV	47.3		fL	39.0 - 56.0
MCH	14.3		pg	12.6 - 16.5
MCHC	30.2		g/dL	28.5 - 37.8
Absolute retic. count	10.9		10^9/L	<=50.0
White Cells	5.3		10^9/L	3.9 - 19.0
Neutrophils (Absolute)	4.40		10^9/L	2.62 - 15.17
Neutrophils	83		%	
Lymphocytes (Absolute)	**0.53**	Low	**10^9/L**	**0.85 - 5.85**
Lymphocytes	10		%	
Monocytes (Absolute)	0.27		10^9/L	0.04 - 0.53
Monocytes	5		%	
Eosinophils (Absolute)	0.11		10^9/L	0.09 - 2.18
Eosinophils	2		%	
Platelet count	**98**	Low	**10^9/L**	**155 - 641**
Analyser ID	Results generated by SYSMEX XT2000 (67683)			
Morphological Assessment :	No morphological abnormalities detected in red blood cell			
	No abnormal white cells seen.			
	Estimation of free platelets		6 platelets seen per HPF	
	Platelet clumps are seen.			

120 x10^9/L

BSH, 7yo, M, “Gunner” (ref. interval **Platelets=200–700 x 10^9/L)**



Blood smear – the emergency patient

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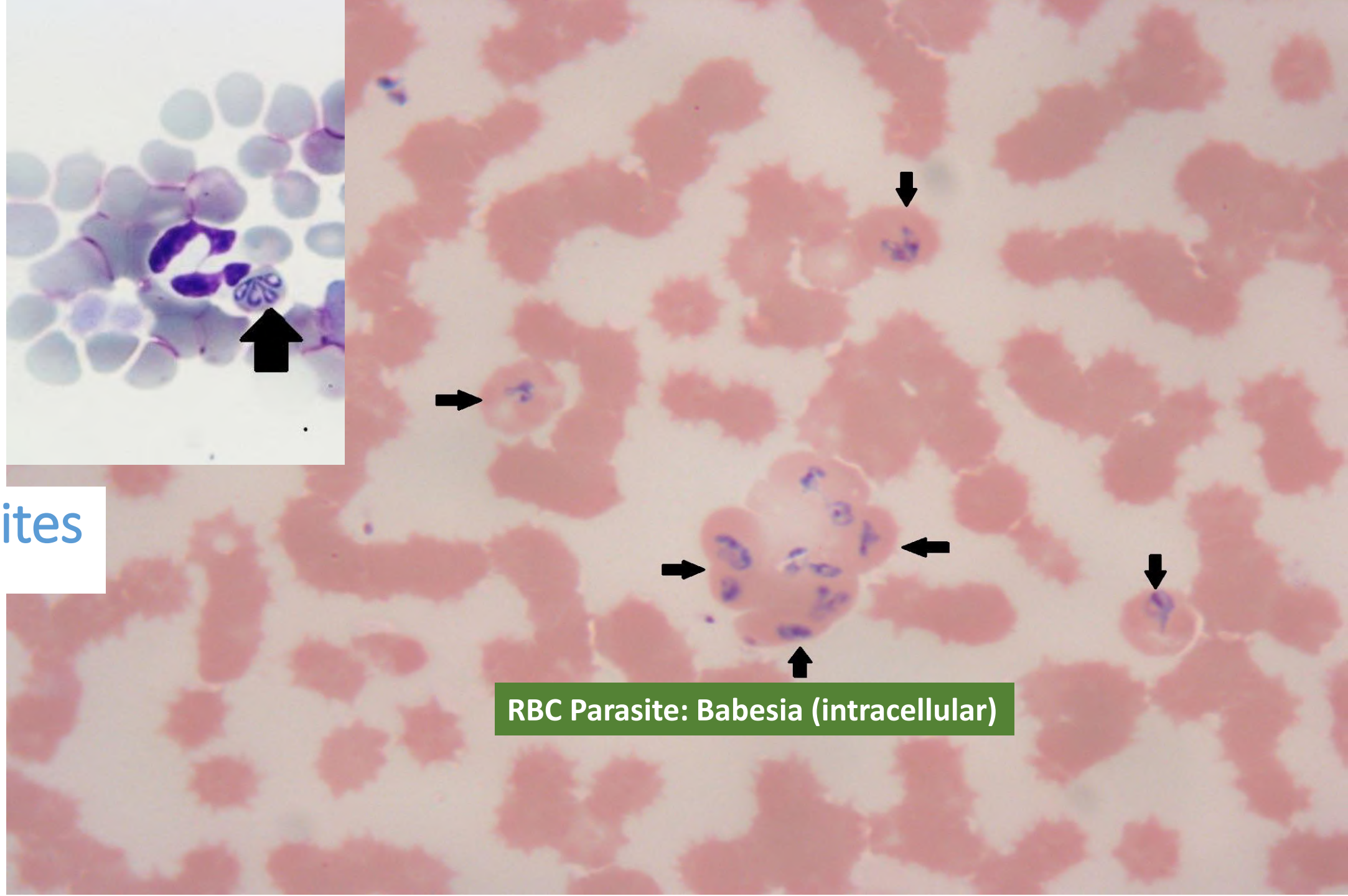
- Is the patient Thrombocytopenic?
- **Any parasites present?**

The emergency patient – Blood parasites

- Can be seen free, on/in RBCs, WBCs
- NOT always present
- NOT always easy to detect

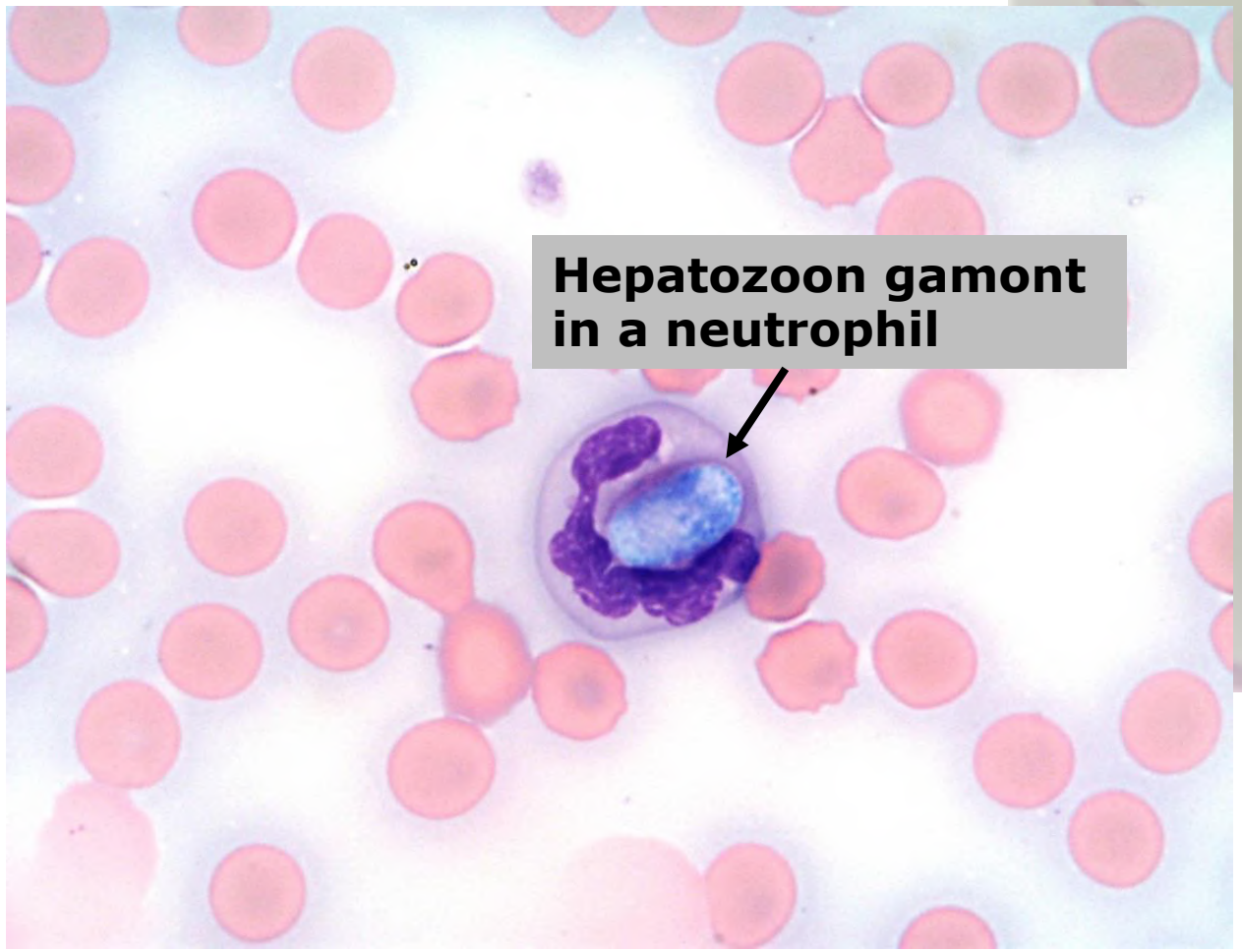
RBC Parasites

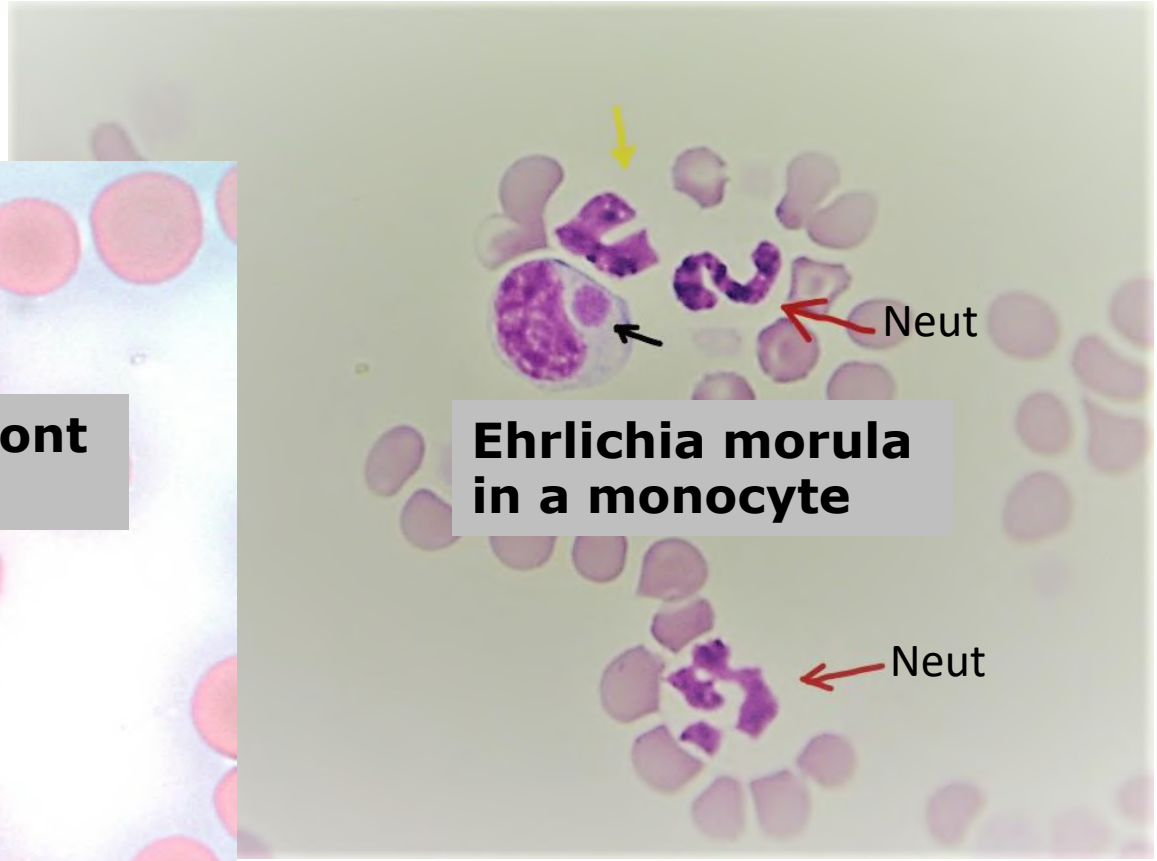


RBC Parasite: Babesia (intracellular)

WBC Parasites

- Not always/Rarely seen on blood smear
- Ehrlichia, Anaplasma, Hepatozoon





Blood smear – the emergency patient -Summary

- **Is the anaemia regenerative?**
  - Anisocytosis, Polychromasia, nRBCs
- **Is the anaemia an IMHA?**
  - Regenerative, Agglutination, Spherocytes, Ghost cells
- **Is there evidence of active overwhelming inflammation?**
  - Immature neutrophils (Left shift), Toxic neutrophils
- **Is a Neoplastic process present?**
  - Atypical/abnormal cells, Blasts (large, prominent nucleolus)
- **Is the patient Thrombocytopenic?**
  - Estimation of platelet count -Manual method
- **Any parasites present?**
  - Dirofilaria
  - Babesia
  - Ehrlichia, Hepatozoon

Tusen takk!

Har du noen spørsmål?