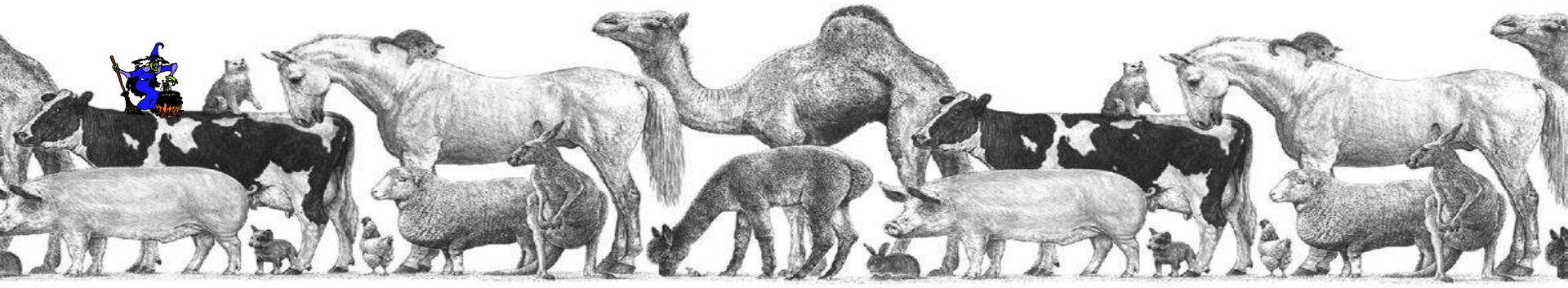
Mystique of the ‘Bone Marrowologist’ – is it based on witchcraft or science?

- What is useful for the referring veterinarian? How can the clinical pathologist convince them that it is based on sound science?
- What are the difficult or controversial parts – I will cherry pick again!
- How does it complement histopathology?

Animal Health Australia

Australian Animal Pathology Standards Program (AAPSP) 2013 Roadshow

Professor Emeritus Paul Canfield, Faculty of Veterinary Science, University of Sydney



What can be learnt from the past?

- Past philosophers, particularly Galen and Celsus, thought that haematopoiesis occurred in the veins *(they were partially right!*). The bone marrow was thought to nourish the bone.
- In 1845 Rudolph Virchow first to describe the microscopic appearance of **leukaemic cells**
- In 1846 John Dalrymple of Dublin examines **bone tissue of a patient with multiple myeloma**

Histopathology becomes dominant over cytology for bone marrow

- Neumann describes nucleated red cells in bone marrow **histological** sections in 1868
- Ehrlich (1879-91) stains and describes blood and bone marrow cells in tissue sections (the latter from necropsy material).
- Pappenheim (1898) uses Romanowsky eosin and methylene blue to stain cells (the real start!)

Paul Ehrlich, 1915 (Wellcome Trust Photographic Library)

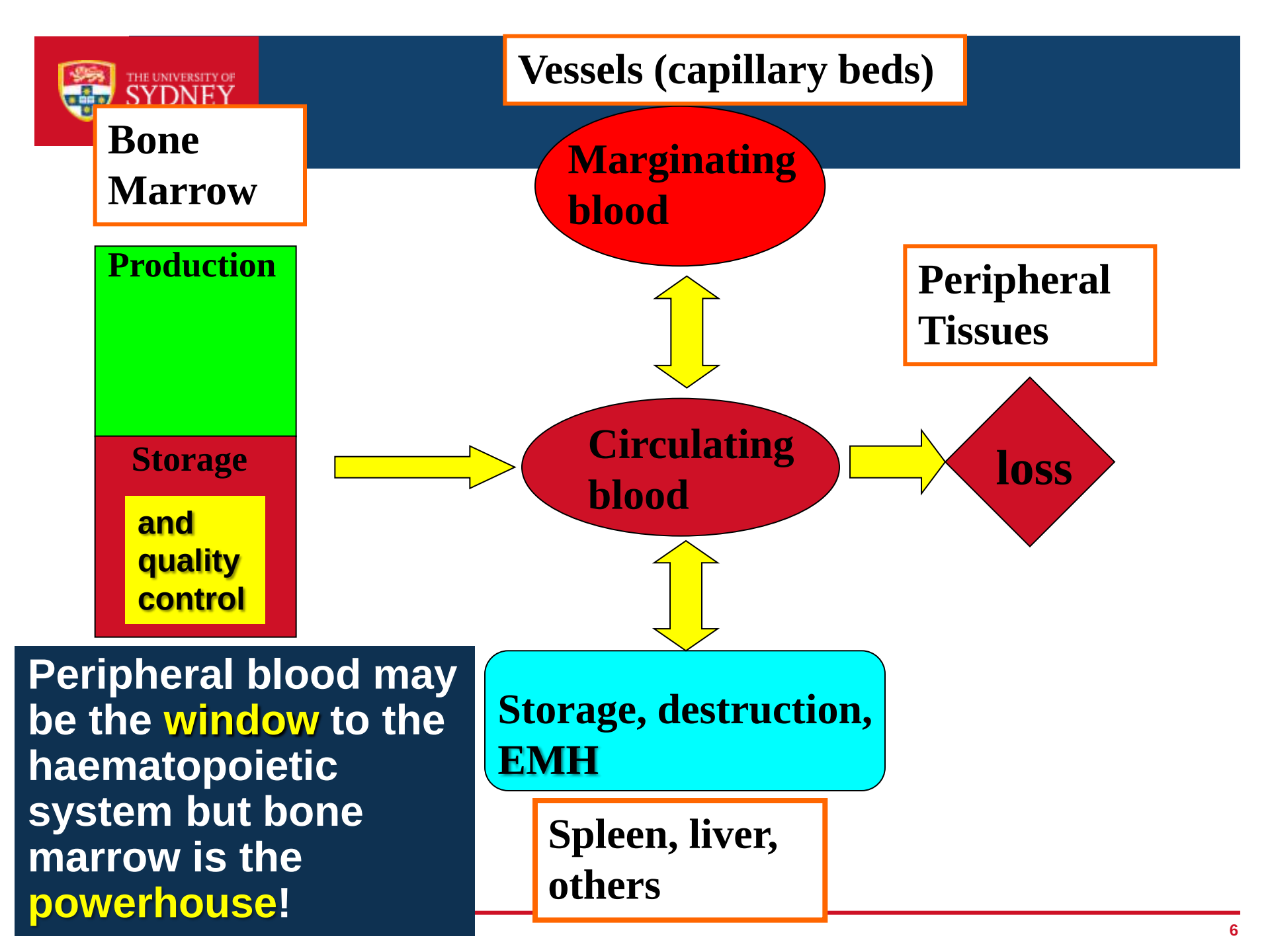
The 20th Century and bone marrow examination

- Bone marrow histopathology from the *living* patient begins in 1903 (surgical trephines)
- Schilling (1925) key for showing the importance of the FBC and the **linkage** of peripheral blood changes with bone marrow
- Up to the 1940's bone marrow continued to be primarily examined by histopathology, but primitive bone marrow aspiration from the living patient began in the 1920's. By the 1950's, human bone marrow aspiration with dedicated aspiration needles (particularly from iliac crest) was commonplace

Veterinary bone marrow aspiration and biopsy

- First published reports in the 1940's of BM aspiration (dog and cat) - Meyer LM and Bloom F (1943). The bone marrow of normal dogs. *Am J Med Sci* 206:637; Sawitsky A and Meyer LM (1947). The bone marrow of normal cats. *J Lab Clin Med* 32:70
- Oscar Schalm a driving force in the 60's and 70's for aspiration and veterinary haematology in general
  - Normal (reference) values based on very few animals!
- Bone marrow biopsy still in vogue and complements the aspirate and is necessary, especially on 'dry taps'

Illinois Sternal Iliac Bone Marrow Aspiration Needles


Vessels (capillary beds)
Bone Marrow
Marginating blood
Production
Storage
and quality control
Peripheral Tissues
Circulating blood
loss
Storage, destruction, EMH
Spleen, liver, others
Peripheral blood may be the window to the haematopoietic system but bone marrow is the powerhouse!

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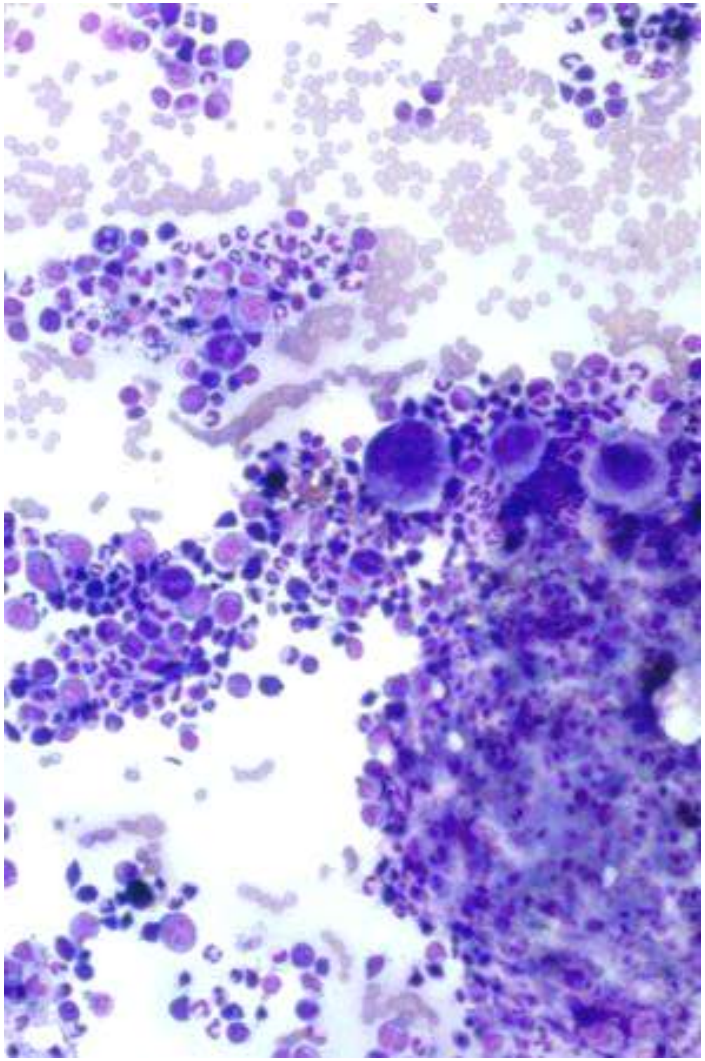
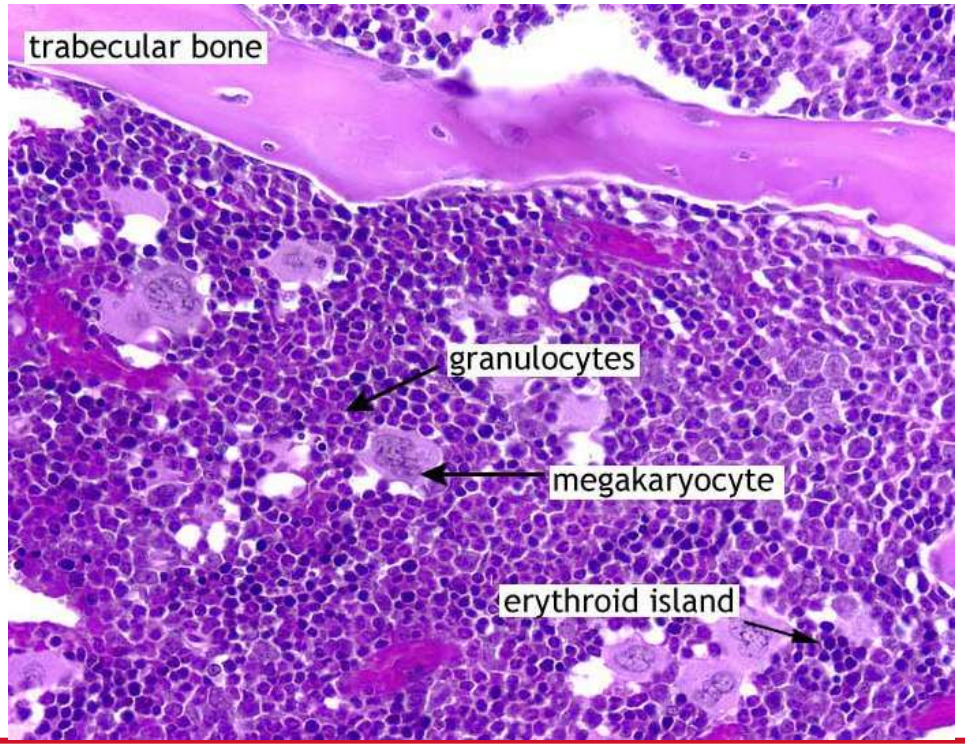
Histopathological and cytological examination of bone marrow are complementary, but why and how?





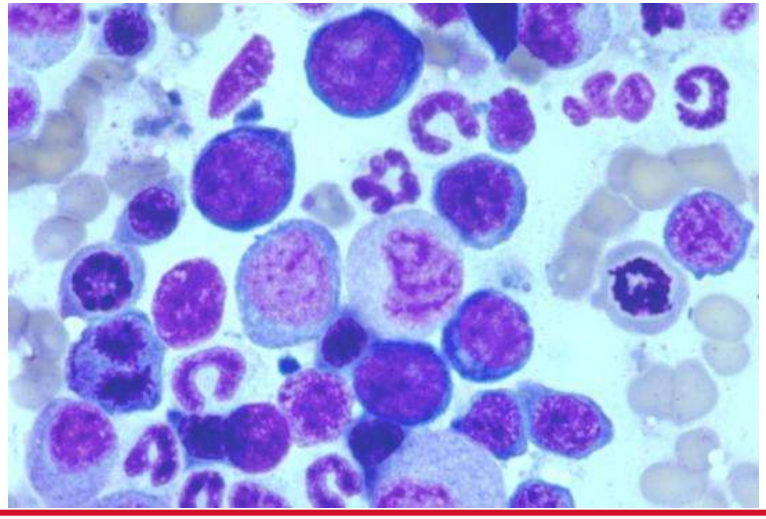
Complementary

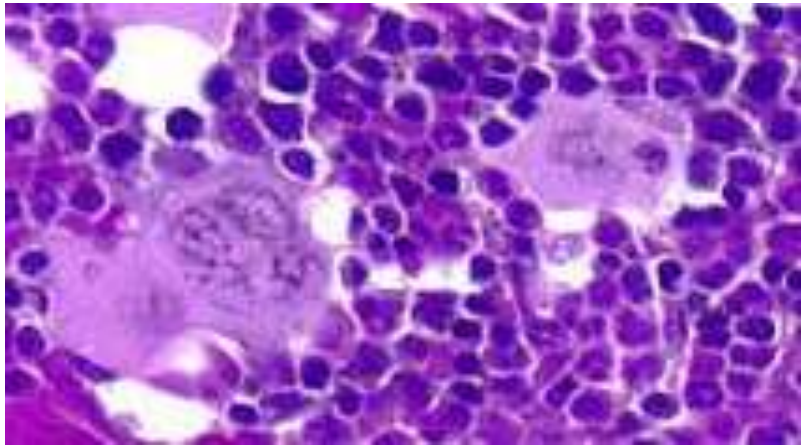
Haemic cytopathology

- **Individual cell identification under 100X magnification (the numbers!)**
- **Overall particle assessment**

Histopathology

- Bone marrow biopsy for **architecture, architecture, architecture**
- **May take Spleen and liver** for assessment of EMH, destruction and infiltration

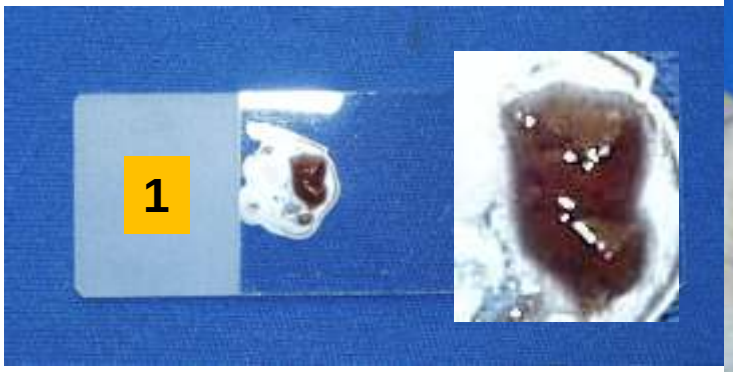


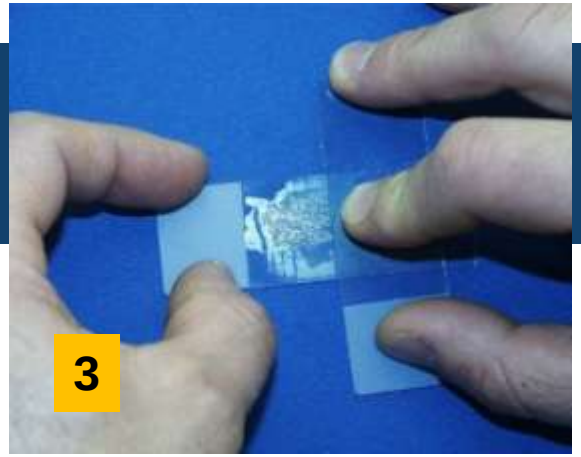


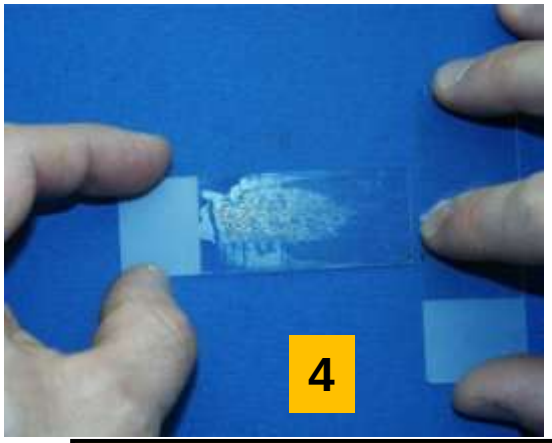
Collection and analysis of the bone marrow aspirate. Is the haematologist (haemic cytopathologist) a help or a hindrance?

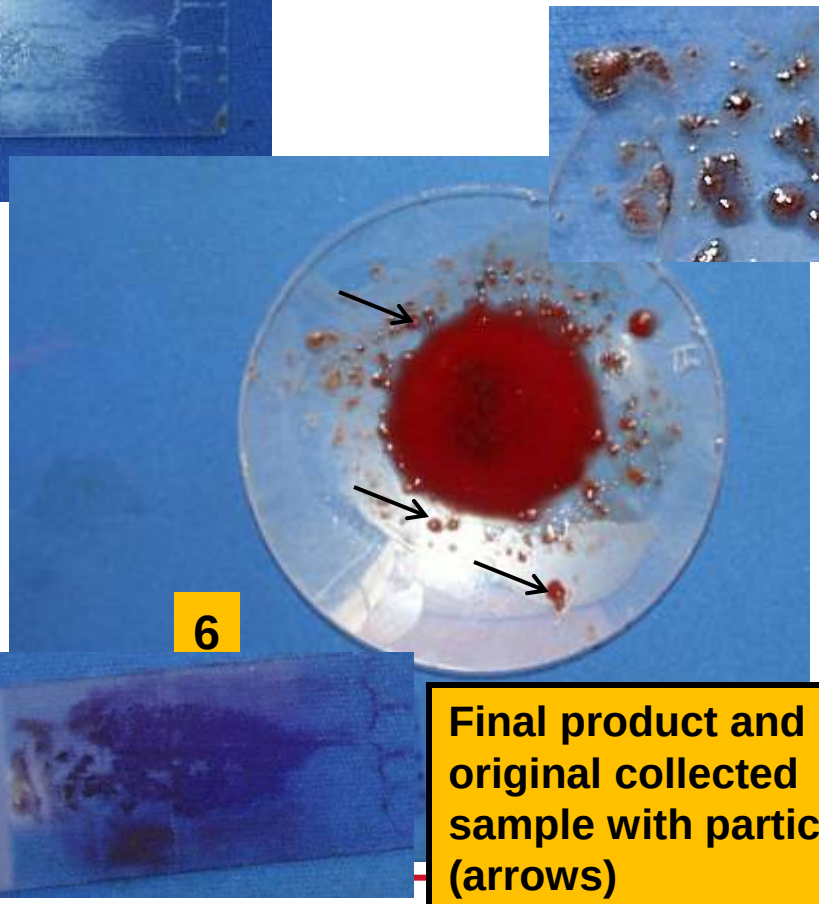
- **My experience:** common reasons for presentation to the laboratory (what is your experience?):

  - **Generally used to follow up mostly persistent peripheral blood changes that cannot be explained simply**
    - -penias, -philias or -cytoses, abnormal cells (neoplastic versus reactive)
    - Adjunct to the investigation of lymphosarcoma (staging) or multiple myeloma
  - **Anaemia**
    - Rarely in regenerative anaemia (perhaps horse?)
    - Useful in unexplained, persistent non-regenerative anaemias (or regenerative anaemias that become non-regenerative)
    - Inappropriate ‘normoblastaemia’
  - **Iron studies**
    - Iron deficiency; iron constipation, iron overload









Making bone marrow smears – If only all the samples were this good!
What about 'buffy coat' preparations of BM?

Final product and original collected sample with particles (arrows)

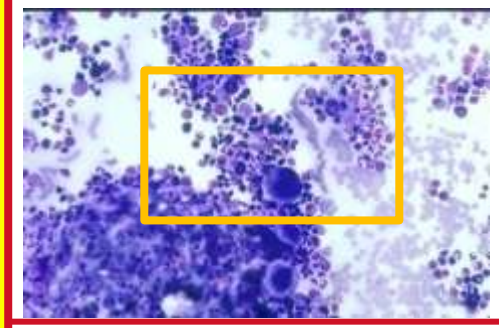
Bone marrow examination - What does it provide (easily)?

- Can do complementary histopathological and cytological examination
- Can link the FBC changes with BM changes (Mantra: **always interpret BM in conjunction with FBC –** *remember Schilling 1925*!)
- For BM cytological examination can determine:
  - proportions of **myeloid and erythroid cells** and their relative **%s of proliferating and maturing pools**
  - percentages of other cells (eg megakaryocytes**)**
  - presence of abnormal cells
  - evidence of destruction and iron storage
- Can you think of other uses? What is useful for the clinician?

How do I do it and how does that compare to how you do it?

- Some haemic cytologists will just provide an overview (‘big picture’ – saves time and suits some personalities)
- Some will separate every type of cell (‘anally retentive’?) and give lots of numbers but give little overview.
- Some will focus on what is useful to the clinician in the count and combine it with an overview (the empathic report)

I look at the edge of a spread particle and differentiate about 500 cells (roughly into proliferating and maturing E and M groups; other cell types (including cells in mitosis and macrophages) . I also count megakaryocytes per particle



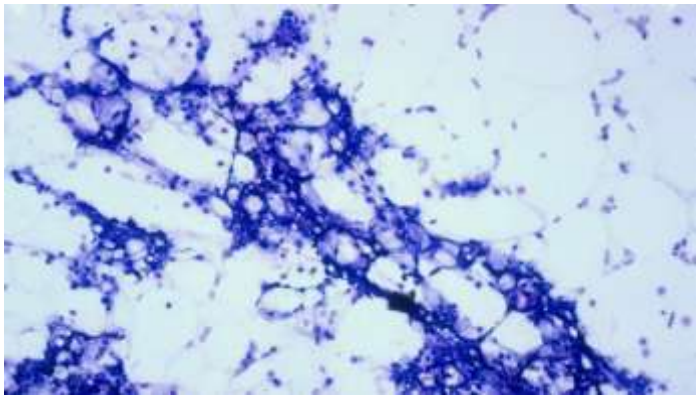
Keep it quiet but -

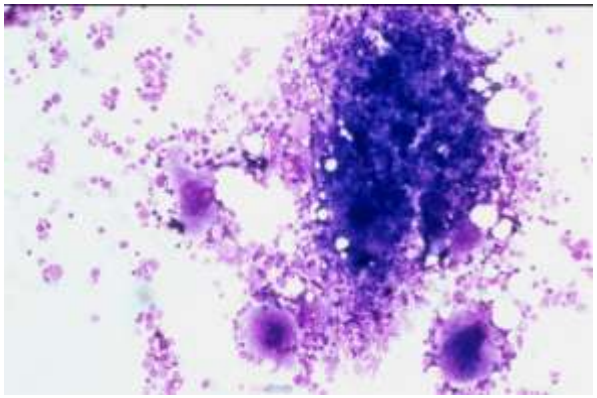
I don't believe there is any clinical pathologist who can truly distinguish every cell into particular categories – we all guess on some cells!

Most clinical pathologists start counting 500 cells but few maintain those numbers as they age!

What affects the count, apart from the clinical pathologist?

- Site of collection (what site do you prefer)?
  - Accessibility a key factor for a particular species
  - Depends on if BM is diffusely affected (bone to bone variation?)
  - Fatty samples can affect ‘spreadability’ and hence counting
    - nb amount of fat affected by site, age and disease





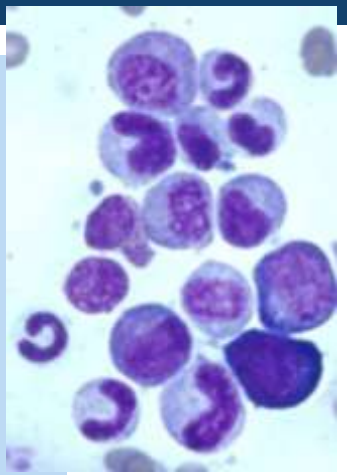
What's the limitation of the M:E ratio?

- M:E ratio (**RELATIVE** numbers of granulocytes [mainly neutrophils and to a lesser extent eosinophils] and erythroid cells). Expressed as a value (number of granulocytes to one erythroid cell eg 1.2 or 1.2:1)
  - Quoted reference values for M:E are extremely variable and very wide!
  - Peripheral blood contamination can affect if –philias or –cytoses present

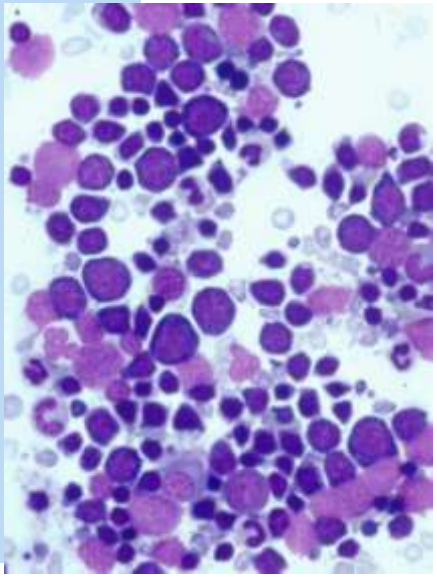
Species	M:E ratio Reference Values (composite from publications)
Dog	1.0-2.0:1
Cat	1.2-2.0
Horse	0.5-2.4
Cow and Goat	0.5-1.6 (usually <1.0 in cattle)
Sheep and rabbit	0.77 to 1.68 (1.0 average)
Pig	1.7-2.0 (Minipigs 0.7-2.1)
Mouse and rat	0.8-2.8
Rhesus macaque	0.8-1.97
Alpaca	0.47-1.0
Birds	1.0-1.2

Reptiles values not found?

M:E alteration – interpretation difficult without the whole picture on blood



- Increased M:E ratio:
  - Myeloid hyperplasia and/or erythroid hypoplasia
    - Increased granulocytes (inflammation or neoplasia - peripheral blood important)
    - Decreased erythroid (intramedullary IMHA and non-regen anaemias)
    - A combination of both
- Decreased ratio
  - Myeloid hypoplasia and/or erythroid hyperplasia
    - Decreased granulocytes (transient or persistent depression and neoplasia)
    - Increased erythroid (regen anaemia; early iron deficiency?)
    - A combination of both



Orderliness of maturation – how useful? Numbers and appearance!

- **Proliferating pool** versus **maturating pool** %s for both myeloid and erythroid (mitoses placed in proliferating pool)
- Increased myeloid/erythroid proliferating pool means good marrow response to usage, loss or destruction *unless the cells are dysplastic or neoplastic!*
- Disorderly maturation, or maturation arrest – think chemical derangements or IM destruction, as well as neoplasia

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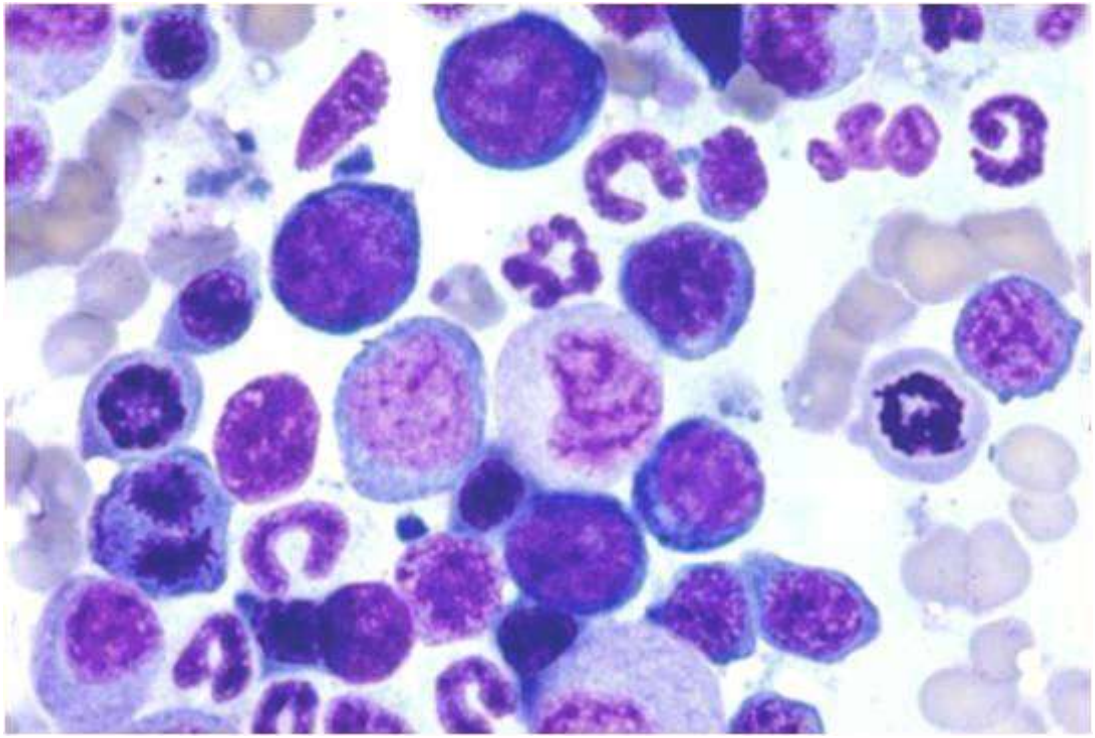
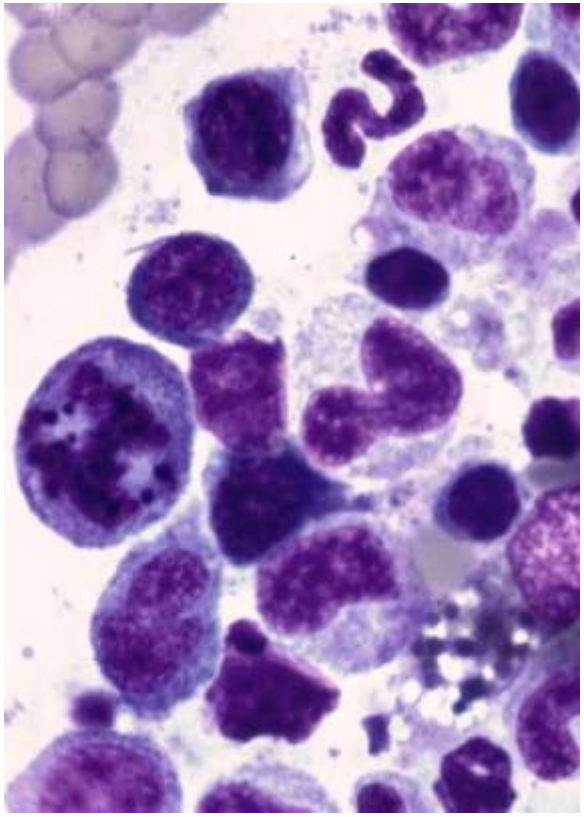
Need to combine M:E ratio and proliferating pools percentages with cell morphology to get the best interpretation for the clinician

Mitotic cell %s complement proliferating pool assessment - as long as you can recognise the cell line!



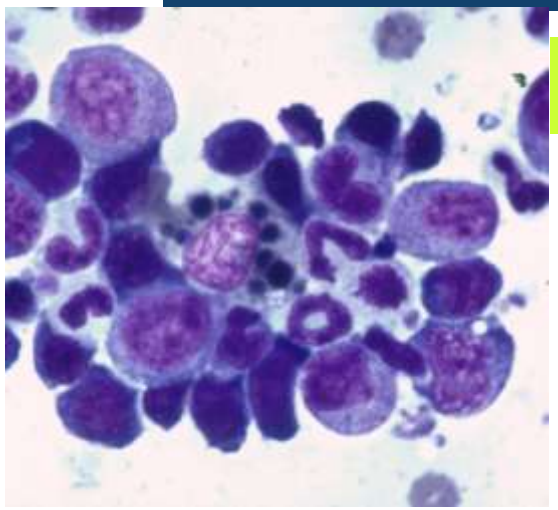


Can you identify the cell lines for these mitoses?

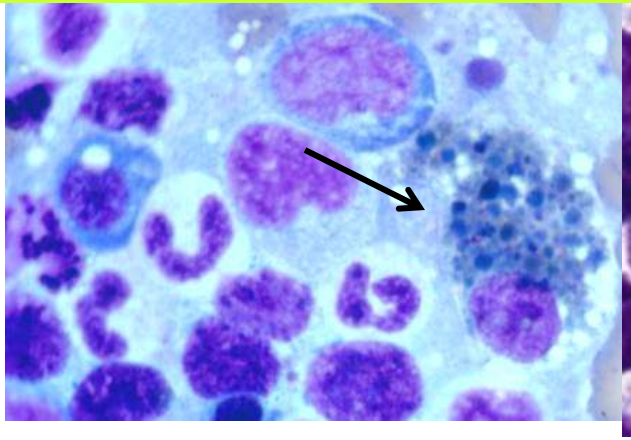
Assessing for intramedullary destruction of erythroid cells

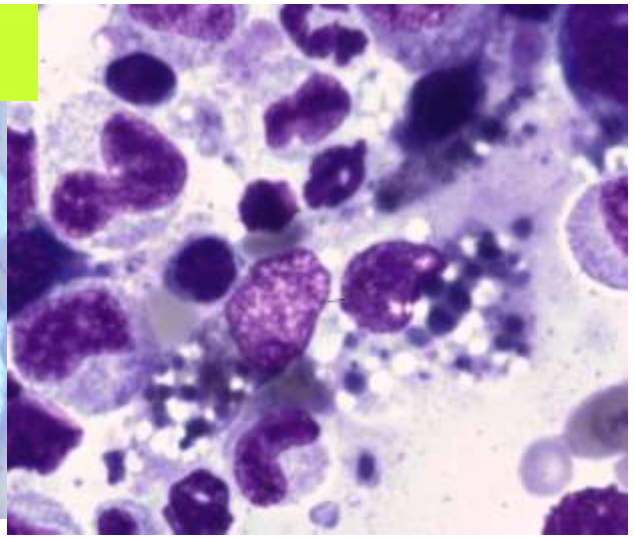
- This may occur when you are considering abnormal or excessive production or you are considering immune-mediated intramedullary destruction
- M:E and E proliferating pool still important, but may also add **macrophage activity (% of total cells – usually <1%) and iron stores**

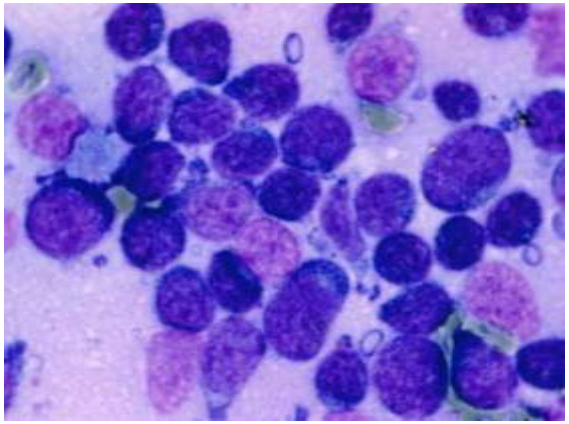
Disorders of the Erythron



The macrophage







Proliferating pool

Perls stain for iron (haemosiderin)

Useful in the cat and cattle?

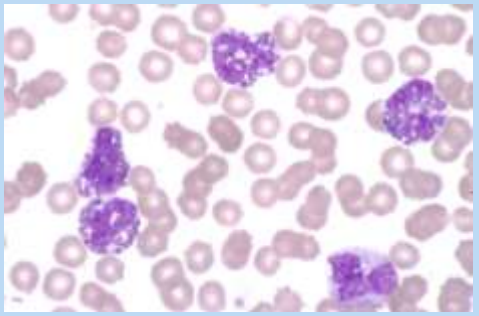
One YO Maltese terrier with non-regen, microcytic anaemia (PCV 0.17, retics 35 x 10^9/L; MCV 39) and thrombocytosis (700 x 10^9/L). WBC count normal.

Bone Marrow Report **1. Detect, Describe**	**Ref Values**
Numerous particles in sample	
M:E Ratio – 0.25:1	1.0-2:1
Myeloid Proliferating Pool % - 17	Up to 20%
Erythroid Proliferating Pool% - 40	Up to 10%
Other: 15-25 megakaryocytes per particle -Erythroid arrest at late normoblast state? -Perl's stain for iron was negative	5-10
2. Deduce (Interpretation)	

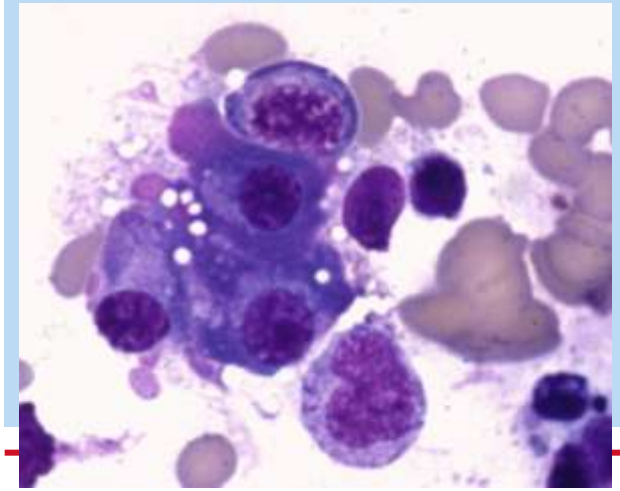
there is erythroid hyperplasia with apparent arrest; there is also increased thrombocytopoiesis. Considering the FBC, history and lack of iron detected in bone marrow, I would suggest iron deficiency as a possibility. Does the dog have evidence of chronic external haemorrhage?

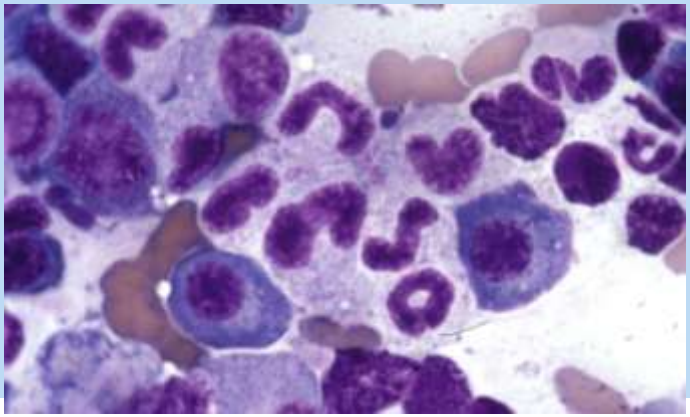
What else can you count in the bone marrow smears?

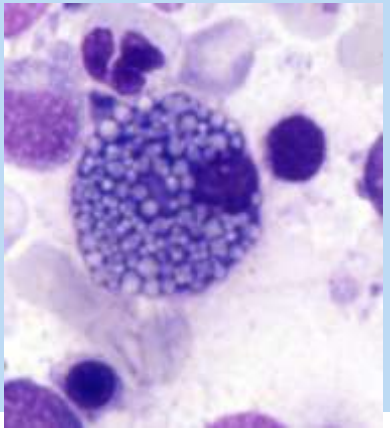
- Eosinophils, monocytes – rarely useful unless you suspect distinct proliferative disorders



- Lymphocytes and plasma cells – immune-mediated destruction? and neoplasia (morphology important)







Three YO Rottweiler with non-regen anaemia (PCV 0.21 L/L; reticulocytes 60 x 10^9/L) and mild leukocytosis (15 x 10^9/L)

Bone Marrow Report **1. Detect, Describe**	**Ref Values**
Adequate particles in sample	
M:E Ratio – 0.4:1	1.0-2.0:1
Myeloid Proliferating Pool % - 21	Up to 20%
Erythroid Proliferating Pool% - 29	Up to 10%
Other: 6-7 megakaryocytes per particle -12% small lymphocytes -7% plasma cells -Numerous macrophages containing iron	5-10 <14% (D) <20% (C) <2% (D &C) <1%
2. Deduce (Interpretation – I think in terms of pathological processes)	

Does this point towards erythroid hyperplasia or myeloid hypoplasia? Is there evidence for increased intramedullary destruction? How do I interpret the increased plasma cells mean? How do I put it all together?

Three YO Rottweiler with non-regen anaemia (PCV 0.21 L/L; reticulocytes 60 x 10^9/L) and leukocytosis (15 x 10^9/L)

Bone Marrow Report 1. Detect, Describe	Ref Values
Adequate particles in sample	
M:E Ratio – 0.4:1	0.75-2:1
Myeloid Proliferating Pool % - 21	Up to 20%
Erythroid Proliferating Pool% - 29	Up to 10%
Other: 6-7 megakaryocytes per particle -12% small lymphocytes -7% plasma cells -Numerous macrophages containing iron	Up to 10 <14% (D) <20% (C) <2% (D &C)
2. Deduce (Interpretation) - there is erythroid hyperplasia associated with increased intramedullary destruction. Myeloid production is adequate. An increase in plasma cells may suggest an immune component. Taking into account the FBC and the history**, I would consider Intramedullary IMHA**	**Coomb's test on bone marrow was positive**

Leukaemias

- Peripheral blood is the starting point – but beware of fluctuations in circulating neoplastic cells!
- BM is necessary to:
  1. Detect myelophthisis
  2. Determine cell type (cytochemistry; immunocytochemistry [immunophenotyping via flow cytometry or ICC on smears or films])
- The pathologist definitely can help with the first, but can struggle with the second (**especially for myeloproliferative disorders where simple morphology is often not enough ie where mature cells are rare**)
- **But the first step is making sure that it is not reactive!**
  - then separating lymphoproliferative from myeloproliferative
  - Then sub-categorizing if necessary

Leukaemia – you definitely can't leave it until after death before diagnosis!

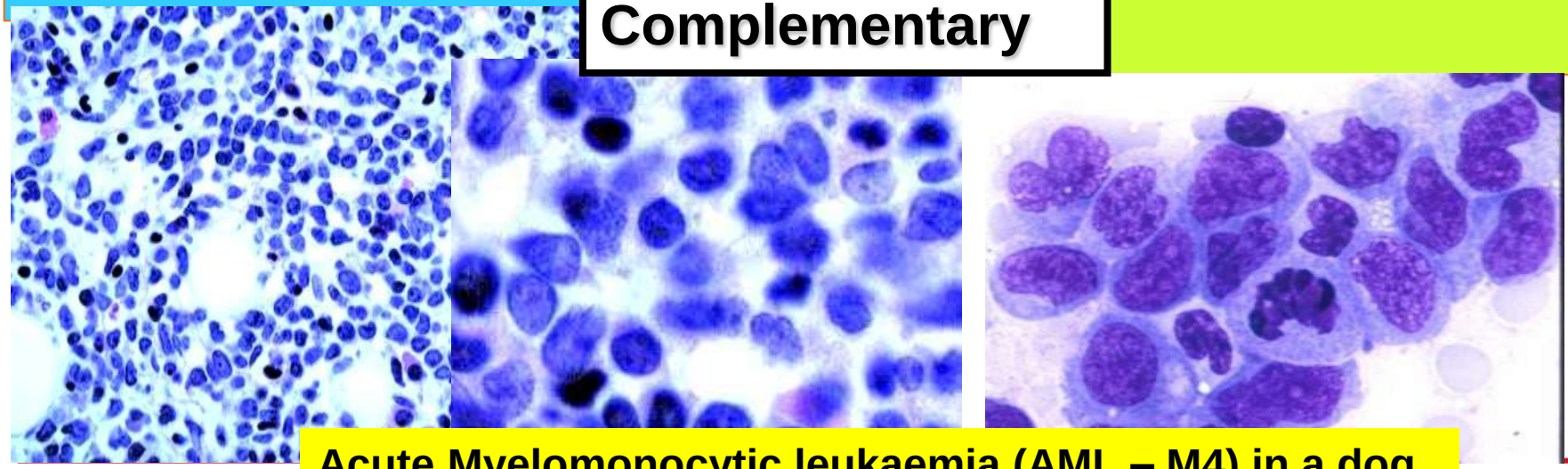
› Haemic cytopathology

- **Peripheral blood** and abnormal cells
- **Bone marrow –**a definite must
- **Cytochemistry and ICC**

› Histopathology

- **Bone marrow** biopsy for loss of architecture
- **Spleen, liver** and other **tissues** for infiltration.
- **IHC**

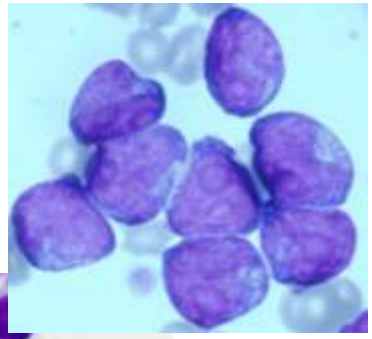
Complementary

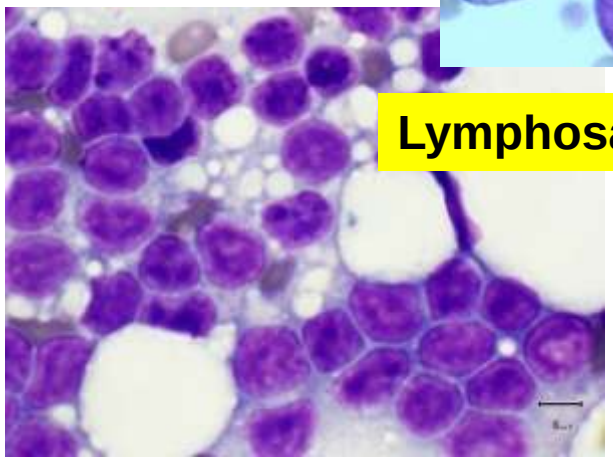


Acute Myelomonocytic leukaemia (AML – M4) in a dog

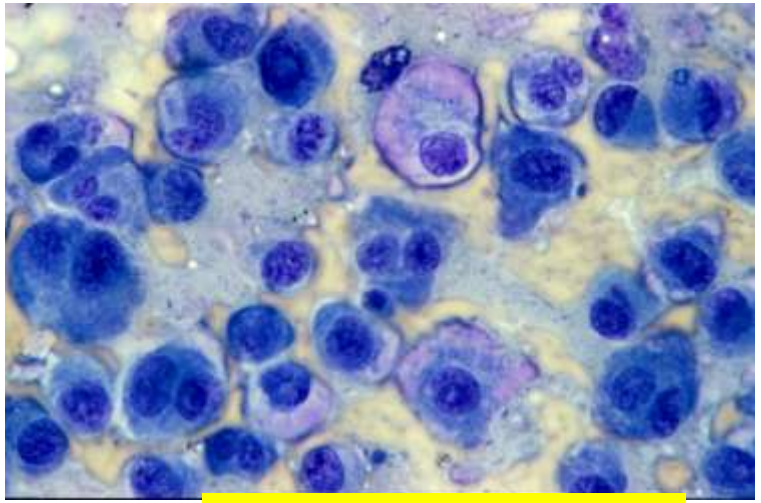
Lymphoproliferative - leukaemia
(Stage 5 LSA; Primary ALL and CLL; Multiple myeloma)

These are far more common than myeloid leukaemias in domestic and wildlife species

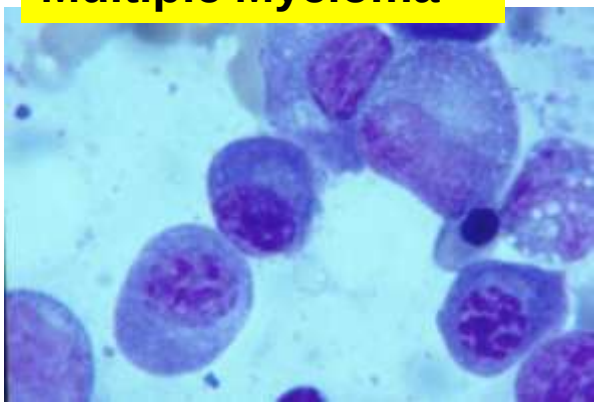




Lymphosarcoma



Multiple Myeloma



LSA is far more common than ALL and CLL; so it stands to reason that lymphoid leukaemia is mostly related to LSA stage 5? Ockham's Razor in action?

Seven YO Rottweiler with non-regen anaemia (PCV 0.12 L/L; reticulocytes 10 x 10^9/L), neutropenia (1.1 x 10^9/L), thrombocytopenia (60 x 10^9/L) and 'atypical' circulating mononuclear cells. There was an abdominal mass

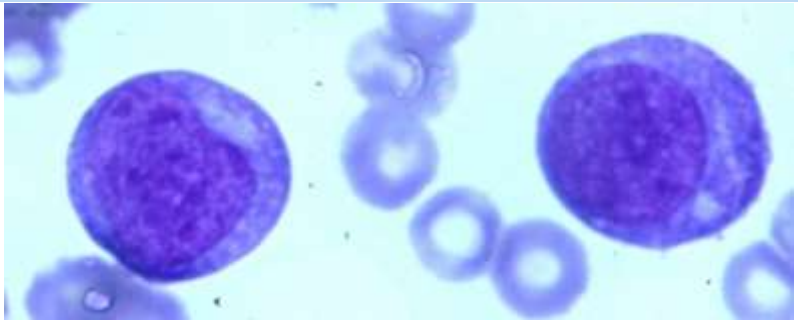
Bone Marrow Report **1. Detect, Describe**	**Ref Values**	
Adequate particles in sample		
M:E Ratio – too few cells to be accurate (occasional NRBC and segmented neutrophil)	1.0-2:1	
Myeloid Proliferating Pool % - ditto	Up to 20%	
Erythroid Proliferating Pool% - ditto	Up to 10%	
Other: <2 megakaryocytes per particle -Numerous (over 90% of cells present) medium to large mononuclear cells with prominent nucleoli, believed to be lymphoid in origin	5-10	**Did I really bother counting 500 cells? Would you?**
2. Deduce (Interpretation)		
Lymphoproliferative disorder (lymphoid leukaemia). If the abdominal mass is lymphoid, then the leukaemia indicates Stage 5 LSA.		

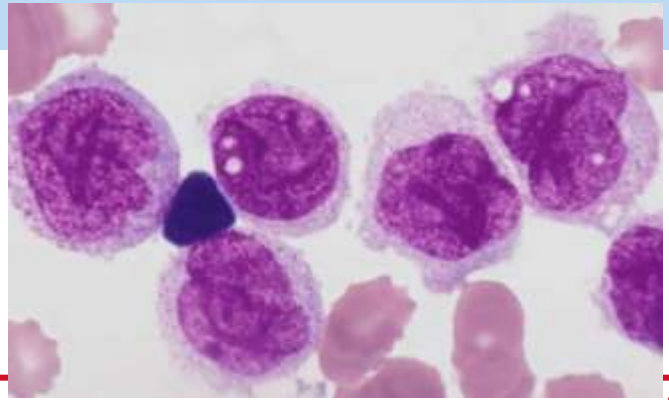
Myeloproliferative disorders

- Uncommon - and thank goodness!
- Adaptation of the FAB (NCIW) system established in the early 90's (mainly for acute leukaemias)
- Still starts with peripheral blood examination
- BM examination essential
- Don't forget splenic/lymph node aspirates!

Myeloproliferative disorders

- **AML (Acute myeloid leukaemia)** – blasts >30% (under review) of E&M cells in BM – AUL plus AML-M1-7 (cover granulocytic, monocytic, erythroid and megakaryocytic leukaemias)
  - Circulating levels extremely variable (remember sub-leukaemia and aleukaemia forms)
- **CML (chronic)** – blasts <30% (under review) with some dysplastic changes, but still 'orderly' production of the cell line affected. **MDS Myelodysplastic disorder)**, affecting any cell line, has significant dysplastic changes with little orderly production (can progress to AML).





Myeloid leukaemia is uncommon (probably less than 10-15% of leukaemias for cat and 5% for dog) – but what is common within the group?

Species	Common types of AML
Cat	M1 and M2 (myeloblastic leukaemias with and without differentiation) most common; M6 (erythroleukaemia) and M6-Er (Erythremic myelosis) next common and almost exclusive to the cat
Dog	M1 and M2 most common; M5a and M5b (monoblastic and monocytic) next most common
Horse	M4 (myelomonocytic most common)

M7 (megakaryocytic) – rare in all domestic species

AML has been recorded rarely in calves (M4, M7 and mast cell leukaemia)

M3 (Promyelocytic only been reported in the pig and rare

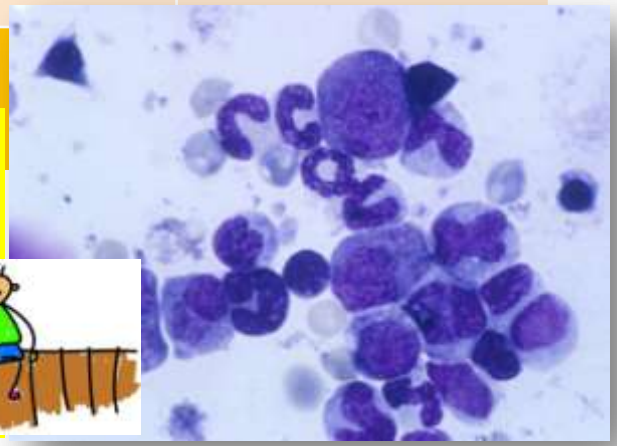
Birds: mostly lymphoid leukaemia, but myeloid (including erythroleukaemia) leukaemia has been described in chickens mainly related to viruses (herpes viruses and retroviruses). Granulocytic sarcomas have been recorded in pet birds.
Reptiles: mostly lymphoid leukaemia, but myeloid (granulocytic and monocytic) leukaemia has been described. No strict classification.
Fish: mostly lymphoid/plasmacytoid leukaemia (retrovirus). BM not applicable (kidney and in some species spleen produce the bulk of cells)

Two year old xbred cat with non-regen anaemia (0.16 L/L) and leukopenia (3.5 x 10^9/L) on peripheral blood

Bone Marrow Report **1. Detect, Describe**	**Ref Values**
Adequate to increased particles in sample but rare megakaryocytes (should be 5-10 per particle on10x objective for dog or cat)	
M:E Ratio - 5:1	1.2-2.0:1
Myeloid Proliferating Pool % - 61	Up to 20%
Erythroid Proliferating Pool% - 1	Up to 10%
Other: some asychrony of neutrophilic maturation	

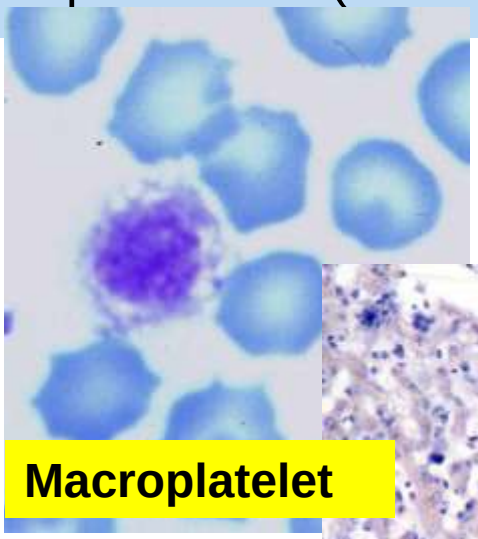
2. Deduce (Interpretation)

There is a distinct possibility of a pre-neoplastic or neoplastic condition primarily affecting the neutrophils, but I cannot completely rule out a damaged BM due to toxicity (with attempts at recovery in the granulocytes).
Suggest repeat FBCs to monitor levels and types of neutrophils circulating plus reticulocyte levels

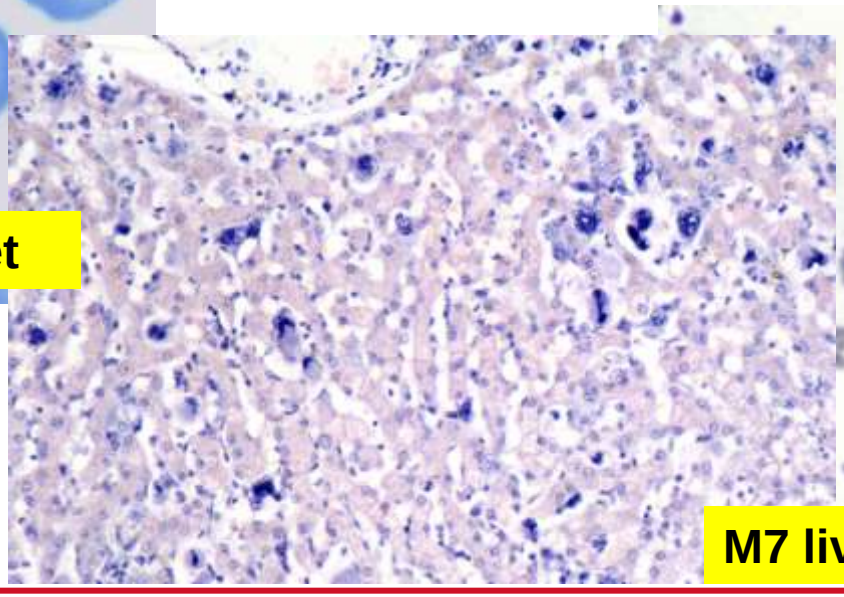


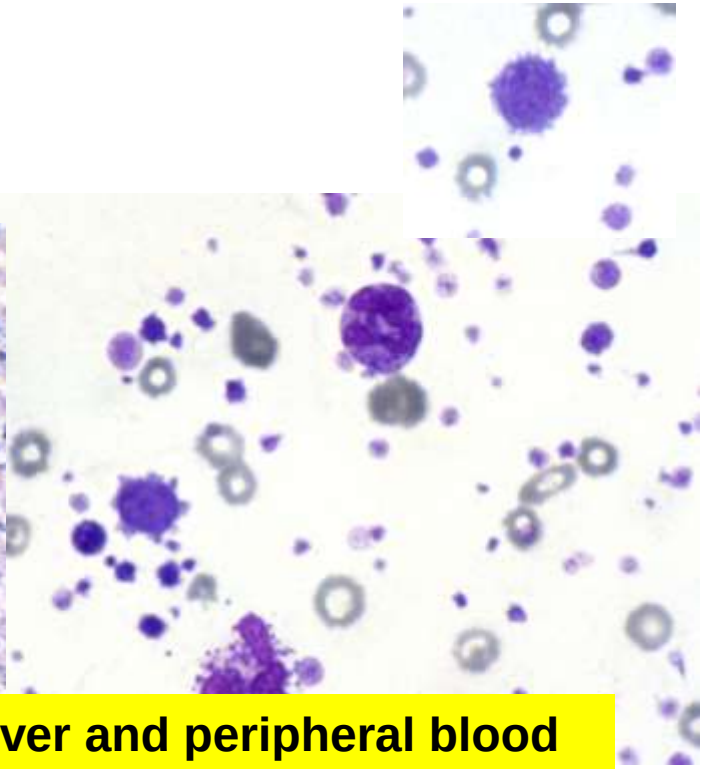
What about megakaryocytes and thrombocytopenia?

- Persistent thrombocytopenia often leads to assessment of BM megakaryocytes
  - Remember, macroplatelets usually suggest enhanced/accelerated production (unless related to neoplasia), but can you really identify them?



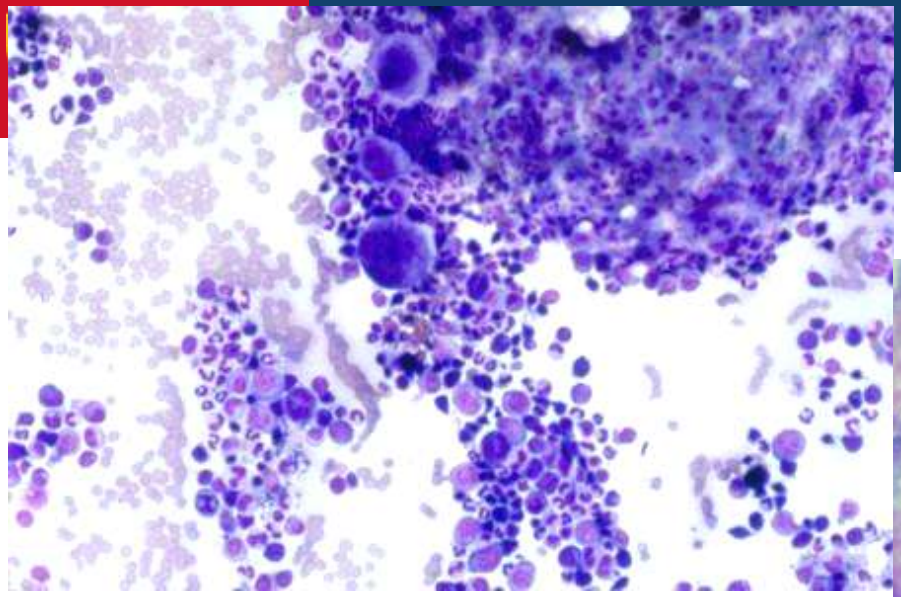
Macroplatelet

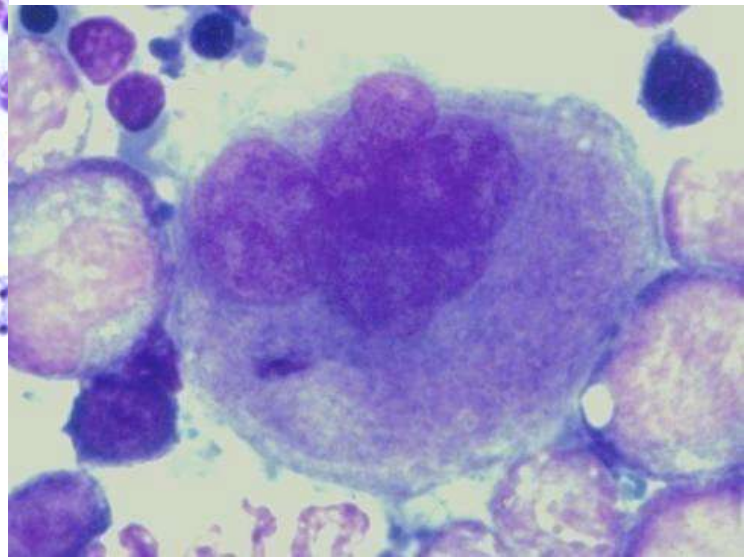


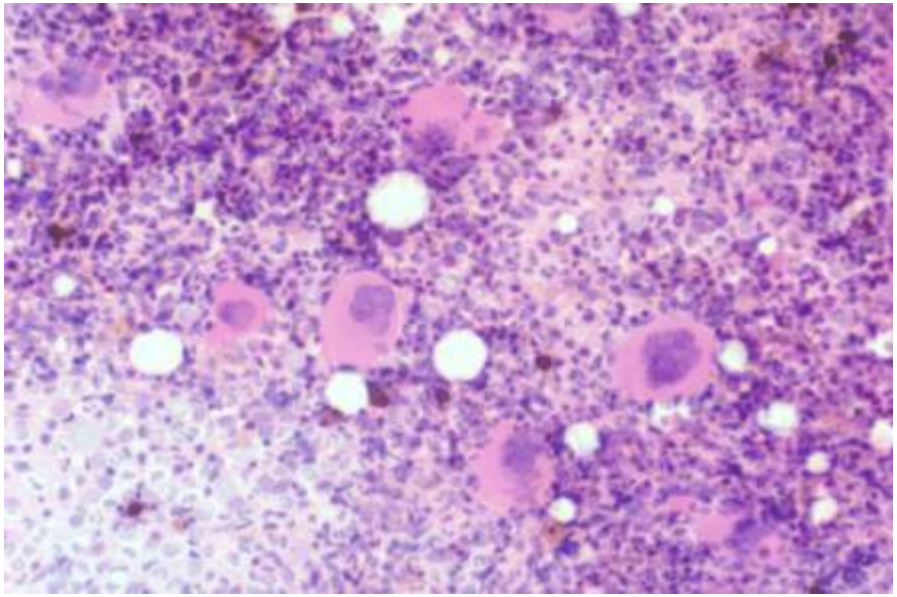


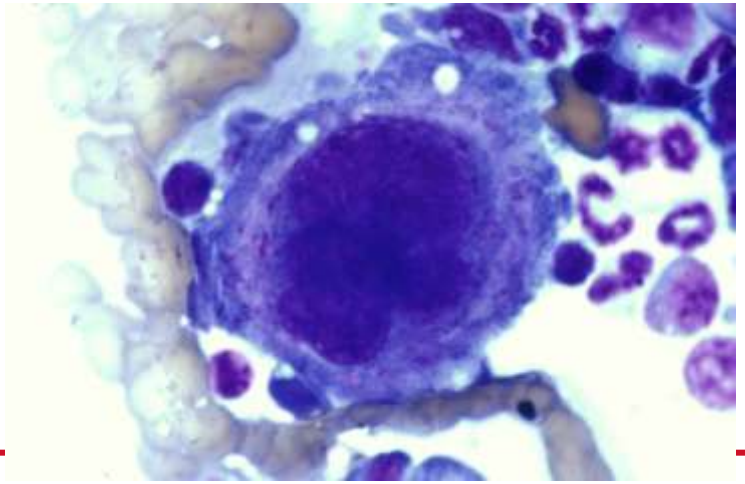
M7 liver and peripheral blood

Assess megakaryocytes within particles on 10X objective (usually between 5-10 for dog and cat)



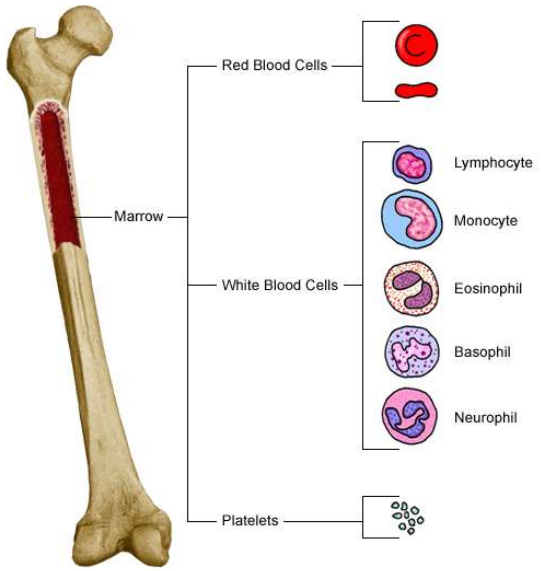






Final remarks

- **Bone Marrow evaluation is an inexact science – all additional information on the case is gratefully received – I tell clients 'enlist rather than test the pathologist!'**
- **Comments on morphology as important, if not more important, than the 'numbers'**
- **FBC essential for BM evaluation**
- **Always consider BM biopsy as well as aspiration evaluation**
- **AML differentiation often very hard without cytochemistry and ICC (and TEM)**



Cases for Discussion

- Can work through the cases on your own, in couples or more
- Use your own style, whether it be pattern recognition and working back or sequential, problem-oriented working forward
- Discussion will be along the lines:
    1. Can a diagnosis be offered and if so what are the key pieces of supporting information?
    2. What results can't be explained by the diagnosis?
    3. If a diagnosis can't be gleaned can you think of a way forward for the referring veterinarian to get a diagnosis (optional)?

An 8-years-old female desexed domestic short hair cat was presented for prolonged polydipsia/polyuria and fluctuating appetite. On presentation, the cat was obese (although it had lost weight according to the owners), not eating and could have been dehydrated. The animal had vomited once. Vital signs were normal.

TEST	SAMPLE	REFERENCE VALUES
ALP IU/L	**54**	<50
ALT IU/L	**92**	<60
Serum protein (biuret) g/L	**93.5**	54-73
Albumin (BCG) g/L	**38.8**	19-38
Globulins g/L	**54.7**	25-50
Total cholesterol mmol/L	**4.9**	1.9-3.9
Glucose mmol/L	**30.5**	3.6-6.6
Urea mmol/L	**11.4**	7.2-10.7
Creatinine µmol/L	110.15	98-180
Calcium mmol/L (uncorrected)	**2.84**	1.7-2.6
Inorganic phosphate mmol/L	1.86	1.3-2.3
Sodium mmol/L	**143**	147-156
Potassium mmol/L	4.2	4-4.6
Chloride mmol/L	**105**	115-130
Bicarbonate (TCO_2) mmol/L	**16.1**	17-24

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	**Marked lipaemia and haemolysis**	Clear
PCV L/L	0.42	0.30-0.45
Plasma protein g/L	**97**	59-78
Haemoglobin g/L	**176**	80-140
Erythrocytes $x10^{12}$/L	9.2	6-10
MCV fL	45	40-45
MCHC g/L	**419**	310-360
MCH pg	**19**	13-17
Leukocytes $x10^9$/L	9.9	8-14
Neutrophils (seg.) $x10^9$/L	7.8	3.8-10.1
Neutrophils (band) $x10^9$/L	0	0-0.4
Lymphocytes $x10^9$/L	**1.2**	1.6-7.0
Monocytes $x10^9$/L	0.1	0.1-0.6
Eosinophils $x10^9$/L	0.8	0.2-1.4
Basophils $x10^9$/L	0	0-0.2
Blood film: no abnormalities		

Urinalysis (cystocentesis)	
Appearance: slight turbidity	pH: 6.5
Colour: light yellow	Glucose: **4+**
Specific gravity: 1.055	Ketones: **trace**
Protein: **trace**	Blood: **2+**
	Bilirubin: -ve
Microscopic findings: much lipid, **40 erythrocytes per HPF.**	

Who uses Corrected Cl = (normal Na/measured Na) x measured Cl (where, normal Na is the midpoint of the Na reference interval)? Detects if *changes in chloride are due to acid-base in balance rather than due to free water changes*.

Likely diagnosis: *Diabetes mellitus* that is progressing towards complications (it is more than a simple diabetic cat but the clinical pathology changes are not really advanced enough to suggest this cat has either hyperglycaemic, hyperosmol syndrome [HHS] or advanced diabetic ketosis [DKA] – only a trace ketonuria and borderline metabolic acidosis; not hyperosmol yet) .
(**Postscript:** the cat was stabilised on insulin and survived for a further 18 months before being killed by a motor vehicle).

Reference: O'Brien TD (2002) Pathogenesis of feline diabetes mellitus *Molecular and Cellular Endocrinology* 197 (1-2):213-219.

Possible reasons for changes: the mild increases in ALP and ALT suggest cholestasis and hepatocellular damage (minor for hepatocellular damage, but it is more difficult to speculate on the degree of cholestasis because in the cat any increase in ALP is significant because of its short half life and lower circulating levels in health). The marked hyperglycaemia is more likely due to an endocrine abnormality (diabetes mellitus). The mild hypercholesterolaemia could be related to liver disease or an endocrinopathy. The cat has borderline metabolic acidosis; the anion gap is slightly increased to 26.1 and suggestive of titration metabolic acidosis; and there is mild ketonuria. The derived osmolality is normal at 306.5 (1.8 x [Na + K] + glucose + urea). The low sodium and chloride are probably related to the polyuria/polydipsia (flushing effect via the kidneys), but hyperlipaemia and hyperproteinaemia could be affecting the assay?. After correcting chloride for the low sodium (Corrected Cl = (normal Na/measured Na) x measured Cl = 111.2 mmol/L) it is still low and may suggest an influence of acid/base imbalance.

while the hyperproteinaemia (both albumin and globulins are increased) is probably due to haemoconcentration (dehydration) or partly due to impact of haemolysis and lipaemia. The calcium is probably high due to the hyperproteinaemia (correction for the hyperalbuminaemia gives a calcium of 2.59; formula for correction is Ca value + [Av Alb – Alb value/40] where average albumin value for the cat is 29), therefore, is of little consequence. The mild azotaemia (urea only) is of little significance and could be due to dehydration and catabolism of protein if the animal is not eating. It is unusual not to get elevation of creatinine in dehydration, but perhaps there are plasma substances interfering with the test method?. The high MCHC and MCH are probably laboratory error directly due to lipaemia and the haemolysis (caused by the lipaemia?) within the tube. The lymphocytopenia is probably due to stress, but the level could be normal for this cat. The trace ketonuria is significant in an adult cat (despite this being urine with a high specific gravity) and could suggest diabetic ketosis (beta hydroxybutyrate is the more sensitive ketone to early diabetes mellitus, but the urine dipstix picks up this ketone body poorly and is more responsive to acetoacetate and acetone; so it is best to determine ketosis by directly measuring blood ketone levels). The high specific gravity is unusual in polyuria, but could be partly influenced by the diabetic condition. The haematuria is likely related to the method of collection, but could be related to something more sinister as it is known that cystitis is common in diabetic animals (further investigation may be warranted).

Likely conclusions, further investigation and implications for management and prognosis?: the pertinent changes suggest a diabetic cat (clinical signs plus hyperglycaemia, hypercholesterolaemia, glucosuria and ketonuria would support a diagnosis of diabetes mellitus) that is progressing towards complications (it is more than a simple diabetic cat but the clinical pathology changes are not really advanced enough to suggest this cat has either hyperglycaemic, hyperosmol syndrome [HHS] or advanced diabetic ketosis [DKA] – only a trace ketonuria and borderline metabolic acidosis; not hyperosmol yet [derived osmolality is 306.9]). The cat was treated with insulin and supportive therapy for the electrolyte and acid-base disturbances.

(**Postscript:** the cat was given insulin and survived for a further 18 months before being killed by a motor vehicle).

A five-years-old Hereford cow was presented with anorexia, weight loss, absent rumen motility, slight diarrhoea, suspected dehydration and depression of two weeks duration. The cow had normal vital signs (eg temperature, heart rate), but on rectal examination had an enlarged left kidney with associated pain.

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.38	0.24-.46
Plasma protein (refract) g/L	**88**	60-85
Fibrinogen g/L	**9**	3-7
Leukocytes $x10^9$/L	10.3	4-12
Neutrophils (seg.) $x10^9$/L	3.9	0.6-4.5
Neutrophils (band) $x10^9$/L	**2.5**	0-0.24
Lymphocytes $x10^9$/L	3.4	2.5-7.5
Monocytes $x10^9$/L	0.2	0-0.8
Eosinophils $x10^9$/L	0.2	0-2.0
Basophils $x10^9$/L	0	0-0.3
Blood film: **mild vacuolation of neutrophil cytoplasm**		

Urinalysis (voided)	PH: 6.0
Appearance: cloudy	Glucose: -ve
Colour: dark yellow	Ketones: -ve
Specific gravity: 1.016	Blood: **2+**
Protein: **1+ (SSA Test)**	Bilirubin: -ve
Microscopic findings: **15-20 erythrocytes and 6-10 leukocytes per high powered field (HPF – 40x objective). Occasional bacteria, 5-8 granular casts per low powered field (LPF – 10x objective)**	

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	280	<300
AST IU/L	110	<130
GGT IU/L	26	<30
GD (GLDH) IU/L	10.2	6.2-13.3
Total serum protein (biuret) g/L	**83**	60-80
Albumin g/L	**39**	28-38
Globulins g/L	**44**	24-40
A:G ratio	0.88	0.84:0.94
Total bilirubin µmol/L	**22**	<10
Glucose mmol/L	**5.1**	3.2-4.0
Urea mmol/L	**40**	5.8-9.2
Creatinine µmol/L	**900**	81-125
Calcium mmol/L	2.8	2.0-3.0
Inorganic phosphate mmol/L	**2.7**	1.4 – 2.6
Sodium mmol/L	148	135-152
Potassium mmol/L	**3.8**	4.0-6.1
Chloride mmol/L	**80**	102-117
Bicarbonate (TCO_2) mmol/L	**37**	24-32
Anion gap	**31.8**	12-22

Likely conclusions*:* there is a strong suggestion of renal disease and dysfunction. Moreover, the renal disease may involve an inflammatory process, possibly due to bacteria, which is having a systemic impact. There is a strong suggestion of upper gastrointestinal stasis (rumen and abomasum) with consequent loss of fluid and acid. This may be secondary to the renal disease or partly independent.

Postscript: the cow was given antibiotics, but became recumbent over the next week. Euthanasia was performed and a necropsy done. The cow was diagnosed with pyelonephritis and hydronephrosis; primarily affecting one kidney (but both were involved).

Possible reasons for changes: considering the clinical history, the hyperproteinaemia may be due to dehydration (especially since both albumin and globulins are equally increased [A:G ratio normal]). The hyperfibrinogenaemia and left shift neutrophilia could indicate inflammatory demand. The toxic changes to neutrophils may be related to inflammation (infection is the most common cause, but not the only cause, of toxic changes). The hyperbilirubinaemia could be due to anorexia (retention hyperbilirubinaemia) since liver enzymes are normal. The hyperglycaemia could be due to corticosteroid release ('stress' perhaps related to the pain?). The moderate azotaemia (both urea and creatinine) could be related to both pre-renal (dehydration, anorexia) and renal factors. The presence of low SpGr in a dehydrated animal and moderate azotaemia strongly suggests renal disease that may be moving to renal failure. The cylindruria suggests renal disease. The haematuria and pyuria may be related to the renal disease or perhaps to lower urogenital tract disease. The pyuria indicates inflammation which could be bacterial in origin. The electrolyte changes can be explained by gut/kidney changes. The hyperphosphataemia could be due to renal failure. The mild hypokalaemia could be due to decreased intake (anorexia), increased GIT loss (diarrhoea; abomasal sequestration), kaliuresis (related to renal failure) and the metabolic alkalosis (shift between ECF K+ and cell H+ to counterbalance decrease of H+ in ECF). The hypochloridaemia (with normal Na) and metabolic alkalosis are probably due to abomasal sequestration (a form of 'internal vomiting' with loss of HCl). The fact that there is an increase in anion gap with the metabolic alkalosis may suggest a mixed acid-base imbalance ('internal vomiting' plus titration MAc due to renal failure and retention of uraemic acids). The paradoxical aciduria (normally, you would expect alkaline urine with MAlk) is probably due to the fact that the low chloride and low potassium interfere with the usual HCO3 secretion and H+ retention in MAlk (eg K+ is reabsorbed at the expense of cellular H+; the low Cl results in Na avidity in the kidney. Na is absorbed in exchange for H+ as Cl cannot be absorbed with the Na+ to maintain electroneutrality– ie blood electrolyte balance becomes more important that metabolic acid-base balance). However, renal disease may be contributing to the final pH, as may be infection (with different results!). The derived osmolality is 313 (1.8 x [Na + K] + glucose + urea), which is high (reference interval is usually around 270-300, but variability for derived osmolality probably extends it).

Likely conclusions*:* *there is a strong suggestion of renal disease and dysfunction. Moreover, the renal disease may involve an inflammatory process, possibly due to bacteria, which is having a systemic impact. There is a strong suggestion of upper gastrointestinal stasis (rumen and abomasum) with consequent loss of fluid and acid. This may be secondary to the renal disease or partly independent.*

(**Postscript:** the cow was destroyed a week later and a necropsy done. The cow was diagnosed with pyelonephritis and hydronephrosis. Primarily affecting one kidney [but both were affected])

Jeremy Allen DAFWA: Illness, ill-thrift, lethargy and deaths of 18 month old SAMM X Merino ewes with 10 dead and 200 affected from a flock of 400. The flock was periodically grazing a barley stubble containing Lesser Loose-strife

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	451	<500
ALT IU/L	**33**	<30
GGT IU/L	14	<67
GD (GLDH) IU/L	0	<20
Total serum protein (biuret) g/L	**78.4**	60-75
Albumin g/L	32.7	28-34
Globulins g/L	**45.7**	30-42
A:G ratio	0.70	0.6-1.1
Haptoglobin mg/mL	**4.48**	<0.6
Total bilirubin µmol/L	0	<15.0
Total conjugated bilirubin µmol/L	0	<5.0
Cholesterol mmol/L	1.95	1.2-2.6
Glycerol mmol/L	0.1	0-0.1
Beta hydroxybutyrate (BHB)	0.21	<0.7
Urea mmol/L	**26.1**	3.3-8.0
Creatinine µmol/L	**295**	50-150
Calcium mmol/L	2.55	2.2-3.0
Inorganic phosphate mmol/L	**2.96**	0.9 – 2.5
Magnesium mmol/L	1.31	0.8 - 1.44
Copper mg/L (plasma only)	**2.41**	0.6-1.1
Iron µmol/L	**30**	33-36
Zinc (plasma only) mg/L	0.71	0.6-1.0
GSHPx in red blood cells U/g Hb	150	>50
Vitamin E mg/L	**0.52**	>1.0
Vitamin A mg/L	**0.22**	Around 0.35

Likely conclusions: the azotaemia with hyperphosphataemia could be related to renal disease. The hyperhaptoglobinaemia and hyperglobulinaemia are likely related to ongoing inflammatory or necrotic conditions (but the latter could be partly due to dehydration). The increased copper could be due to ceruloplasmin elevation in it's role as an acute phase reactant? Vitamin E and A? No evidence for liver damage (see reference)

Necropsy findings: acute renal tubular necrosis, ruminal parakeratosis (ruminal acidosis)
Aetiological diagnosis: Toxic nephropathy; presumed Lesser Loose-strife (*Lythrum hysoppifolia)* nephrotoxicity.
Reference: Lythrum hyssopifolia (lesser loosestrife) poisoning of sheep in Victoria. MJ Lancaster *et al. Aust Vet J* 2009;87:476–479

A two-years-old male dachshund was presented with vomiting and depression of two days duration. On examination, the dog appeared to have acute abdominal pain. There was some suggestion of polyuria and polydipsia for some considerable time prior to this episode.

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	110	<200
Amylase IU/L	**1800**	<1400
Lipase IU/L	**398**	<60
ALP IU/L	**1992**	<110
ALT IU/L	**389**	<60
Total protein (biuret) g/L	**83**	50-70
Albumin g/L	39.5	23-43
Globulins g/L	42.5	27-44
Total cholesterol mmol/L	**12.87**	1.4-7.5
Glucose mmol/L	**7.83**	3.3-6.4
Urea mmol/L	**78**	3.0-10
Creatinine µmol/L	**1049**	105-120
Calcium mmol/L	**1.92**	2.1-2.9
Inorganic phosphate mmol/L	**10.32**	0.8-1.6
Sodium mmol/L	149.2	137-150
Potassium mmol/L	4.2	3.3-4.8
Chloride mmol/L	**97.3**	105-120
Bicarbonate (TCO_2) mmol/L	**13.5**	18-24

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.42	0.37-.50
Plasma protein (refract) g/L	**107**	55-75
Leukocytes $x10^9$/L	**17.3**	7-12
Neutrophils (seg.) $x10^9$/L	**14.7**	4.1-9.4
Neutrophils (band) $x10^9$/L	0	0-0.24
Lymphocytes $x10^9$/L	**0.87**	0.9-3.6
Monocytes $x10^9$/L	**1.73**	0.2-1.0
Eosinophils $x10^9$/L	**0**	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film**: target cells +**		

Urinalysis (cystocentesis)	
Appearance: clear	PH: 6.5
Colour: light yellow	Glucose: **2+**
Specific gravity: 1.011	Ketones: -ve
Protein: **3+**	Blood: **+ve**
	Bilirubin: **trace**
Microscopic findings: occasional struvite crystal, 7 erythrocytes per HPF and less than 1 leukocyte per HPF.	

Possible conclusions: there is enough evidence to suggest that the dog has renal failure. This could be end stage (although no anaemia has been detected; but remember the animal is haemoconcentrated) and related to the previous polyuria and polydipsia. However, it does not explain the acute abdominal pain, the hepatopathy and possible pancreatic necrosis. Therefore, it is likely that an hepatopathy and/or acute pancreatic necrosis have occurred in the short term. The hepatopathy (mainly cholestasis) could be secondary to the acute pancreatic necrosis (bile duct blockage and toxic effects on the hepatocytes).

Postscript: the dog was treated for renal failure and suspected acute pancreatic necrosis. The amylase and lipase continued to rise and the dog developed a neutrophilia with a left shift, probably confirming an acute pancreatic necrosis. Eventually, this was brought under control. The dog was sent home with a poor prognosis due to its chronic renal problem.

Likely reasons for changes: the history and physical examination suggest an acute onset illness, but with an underlying disease process (polyuria and polydipsia). This could suggest two connected disease processes or two separate ones. The high protein is probably due to haemoconcentration via vomiting (this may mean that the PCV is lower than what it seems). The difference between plasma and serum protein could be due to different methods of measurement, but fibrinogen increases may be contributing. The low chloride is also probably due to the vomiting (correcting it for the sodium [Corrected Cl = (normal Na/measured Na) x measured Cl] gives a further reduced value of 93.6 mmol/L and suggests independent change due to acid-base imbalance) . Vomiting usually causes metabolic alkalosis, but this dog has metabolic acidosis with increased anion gap. This suggests an overriding process where acids are retained in the blood stream (hence the increase in the anion gap). Renal failure is the common reason for this. Marked azotaemia could fit in with this, but prerenal factors are probably contributing to this. The increase lipase could be due to pancreatic necrosis, but sometimes can be increased in renal failure and anything else that causes reduced GFR (eg dehydration). The amylase is only mildly elevated and this could be due to pancreatic necrosis (sometimes it is not very consistent) or renal failure/decreased GFR (apparently related to increased formation of macroamylase [globulin bound amylase]) . The increases in ALP and ALT suggest cholestasis and hepatocellular damage respectively. The high cholesterol and target cells could also go along with hepatopathy or acute pancreatic necrosis. Some kidney diseases can also increase cholesterol. The hyperglycaemia could be due to corticosteroid release (the leukogram changes definitely fit in with stress), but hyperglycaemia in dogs due to stress is less common than in the cat. Hyperglycaemia sometimes occurs related to insulin shutdown in acute pancreatic necrosis. The low calcium can sometimes occur in renal failure, but can also occur in acute pancreatic necrosis due to deposition of calcium fatty acids. The high inorganic phosphate could be due to renal failure. The urine suggests a nephropathy (proteinuria and low specific gravity). The haematuria is probably due to the mode of collection. The glucosuria is unusual as it normally doesn't occur until the glucose levels in the blood are above renal threshold. However, it may be related to urinary tubular damage (renal glucosuria).

Possible conclusions: there is enough evidence to suggest that the dog has renal failure. This could be end stage (although no anaemia has been detected; but remember the animal is haemoconcentrated) and related to the previous polyuria and polydipsia. However, it does not explain the acute abdominal pain, the hepatopathy and possible pancreatic necrosis. Therefore, it is likely that an hepatopathy and/or acute pancreatic necrosis have occurred in the short term. The hepatopathy (mainly cholestasis) could be secondary to the acute pancreatic necrosis (bile duct blockage and toxic effects on the hepatocytes).

(**Postscript:** the dog was treated for renal failure and suspected acute pancreatic necrosis. The amylase and lipase continued to rise and the dog developed a neutrophilia with a left shift, probably confirming an acute pancreatic necrosis. Eventually, this was brought under control. The dog was sent home with a poor prognosis due to its chronic renal problem.)