Cytopathology – Selected sites

- What is useful for the referring veterinarian? Value adding for the dermatologist and other specialists?
- What are the difficult or controversial parts – I will cherry pick again!
- Cytopathology definitely complements histopathology for internal organs?

Australian Animal Pathology Standards Program (AAPSP) 2013 Roadshow

The journey continues through the cytopathologist's World of thoughts and illusions!

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

The loneliness of the journey for cytopathologists!

› What can go wrong will go wrong for me – unless I stick to my approach!

'I should have rung my aging mother this morning'

'Will I get through the day with just one coffee?'

Can I deduce a pathological process whilst under pressure?

'I wonder what the accountant wants to talk to me about?'

'How will I pay for the spouse's new car?'

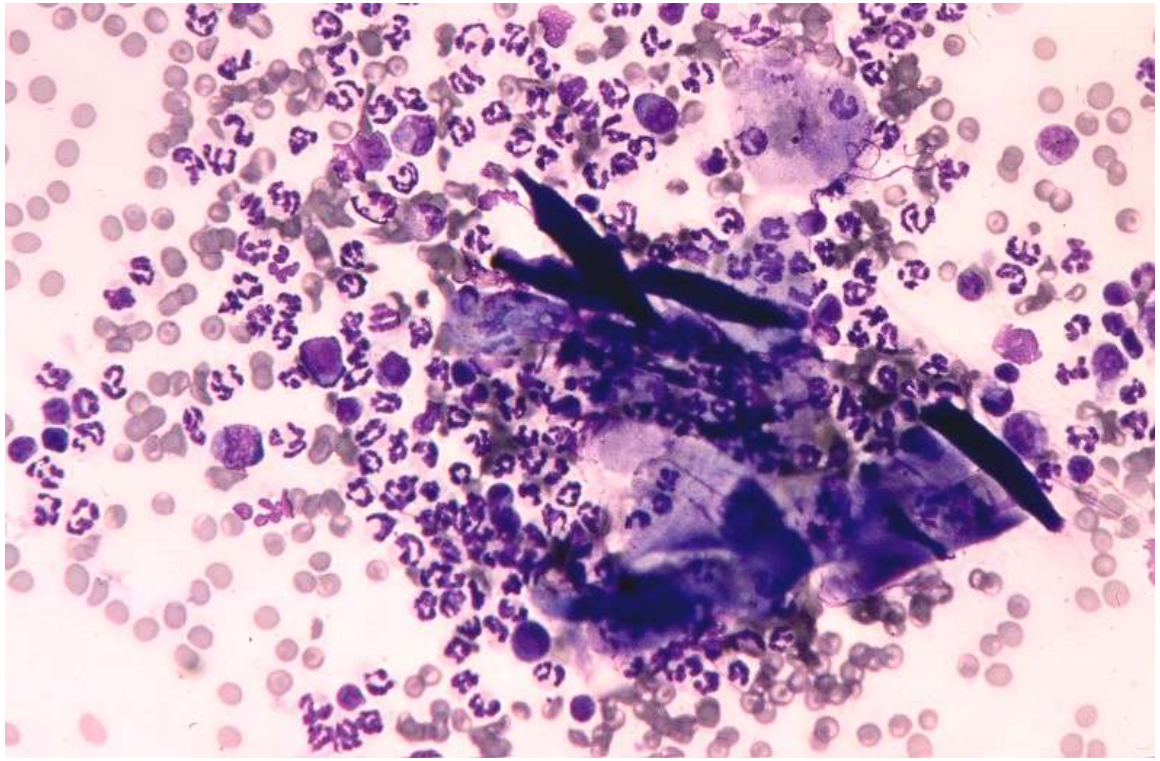
Skin and subcutis

- Who finds scrapings and imprints diagnostic?
- How good are the FNA smears you receive?
- How many of you have the opportunity to check your diagnosis through histopathology feedback?
- Does the Anatomical Pathologist treat you like the poor relation and never talks to you unless he wants something?

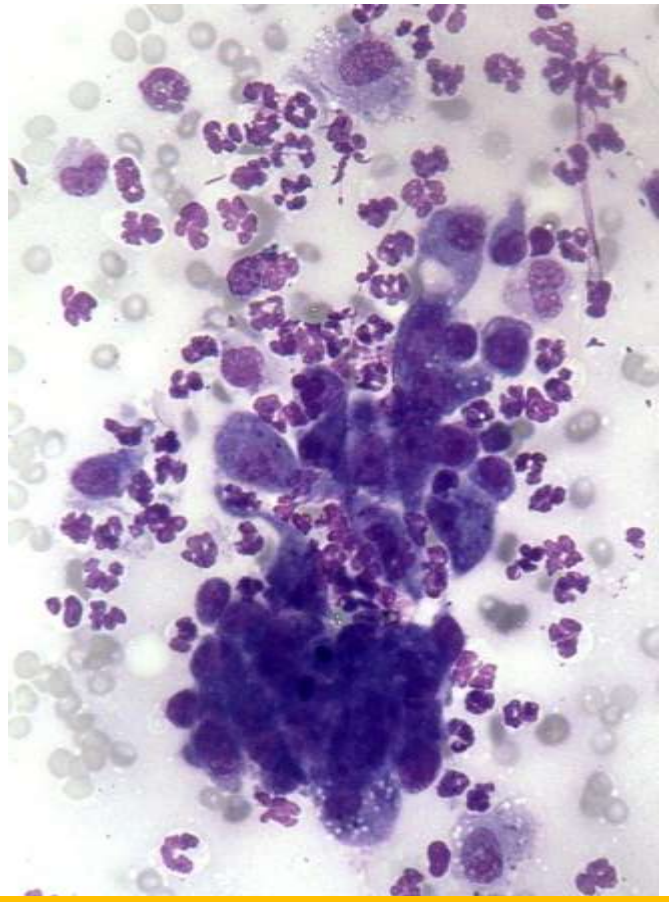
Skin and subcutaneous samples are common for the cytopathologist , so it is fortuitous that correlation with histopathology is highest for these sites (75-90% correlations reported)

Issues I have with cytology of skin and subcutis

Is inflammation masking a tumour?

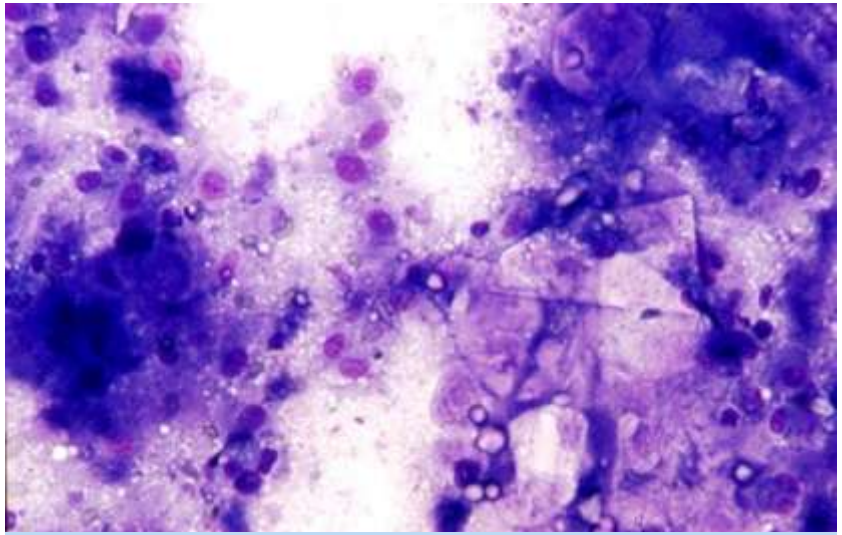


Furunculosis or inflamed adnexal tumour in a dog?

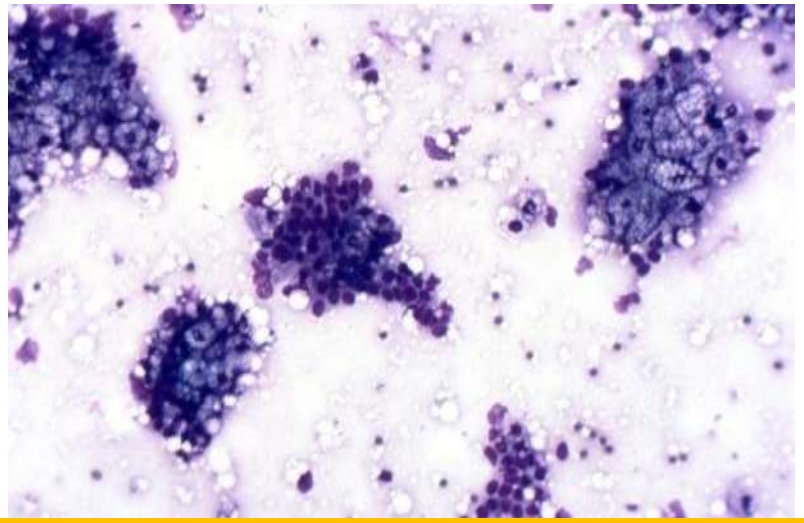


Inflamed perianal carcinoma in a dog or could it be pyogranulomatous dermatitis?

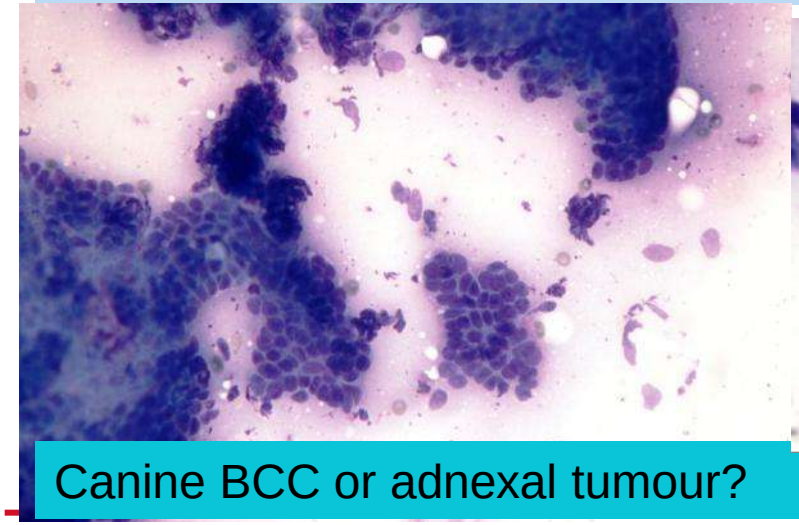
Differentiating adnexal hyperplasia and tumours (including BCC) is difficult for me!



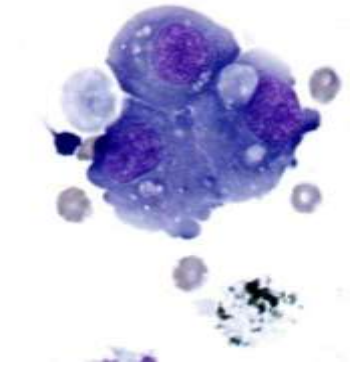
Canine follicular cyst or tumour?



Canine sebaceous tumour or hyperplasia?

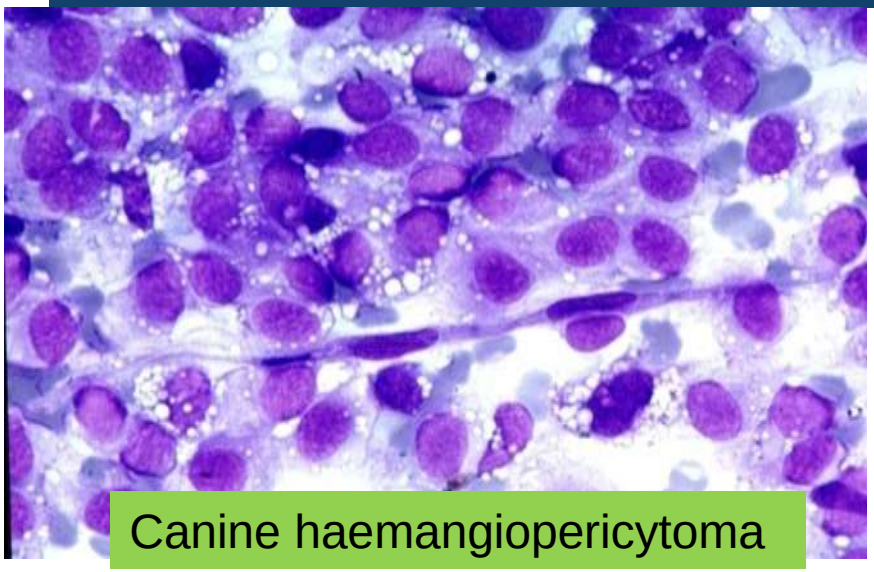


Canine BCC or adnexal tumour?

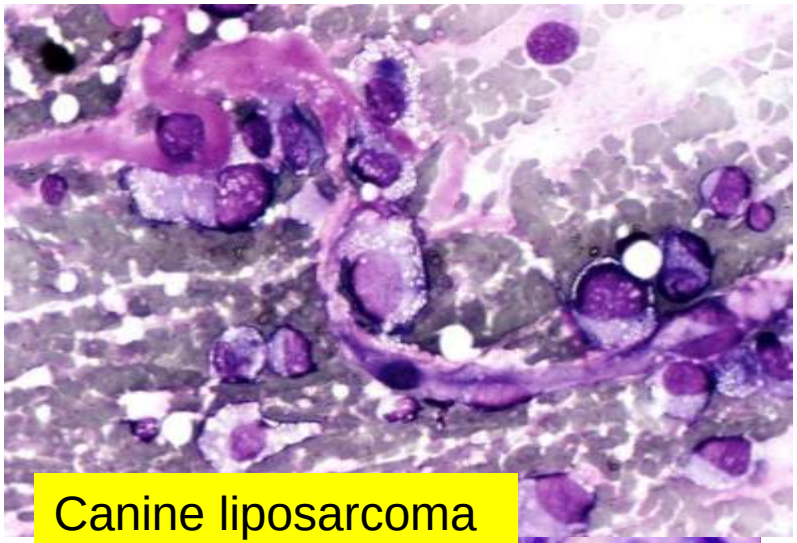


Canine sweat gland (apocrine) carcinoma and cystadenoma (left to right)

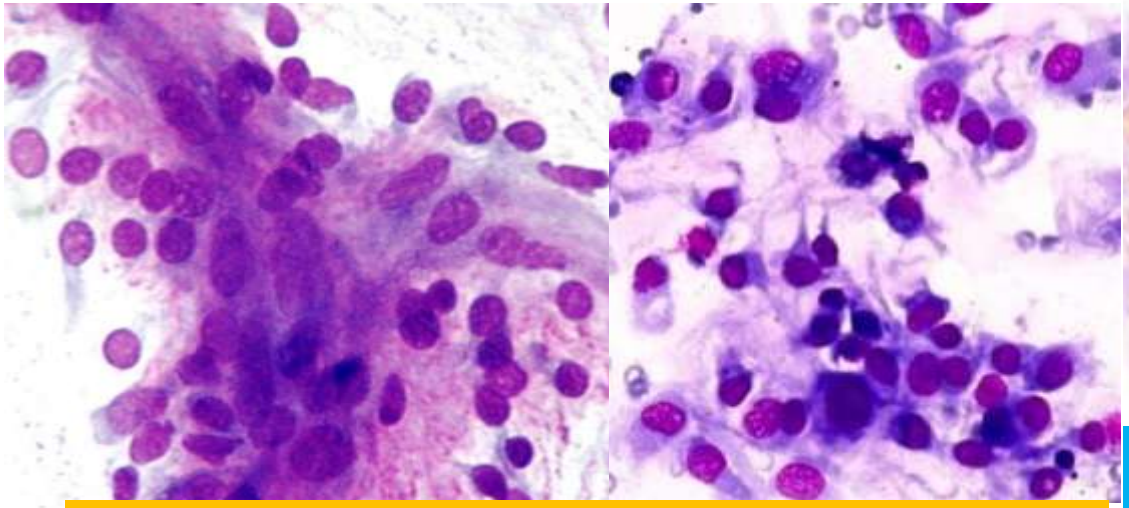
Those soft tissue sarcomas in dogs! (I'd also include equine sarcoid versus fibrosarcoma versus granulation tissue!). How far do you go in diagnosis?



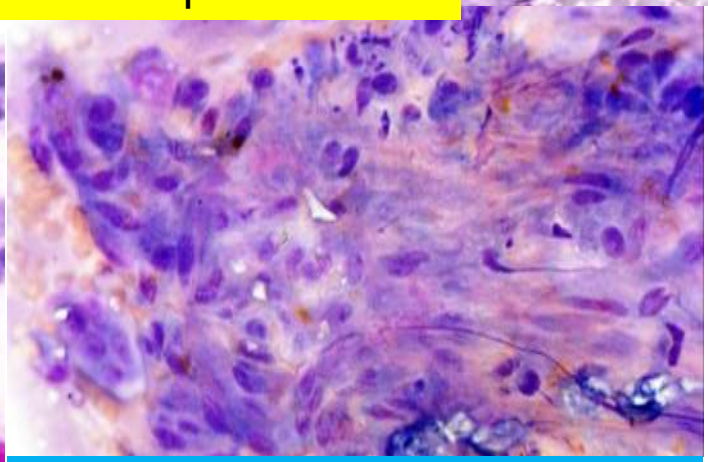
Canine haemangiopericytoma



Canine liposarcoma

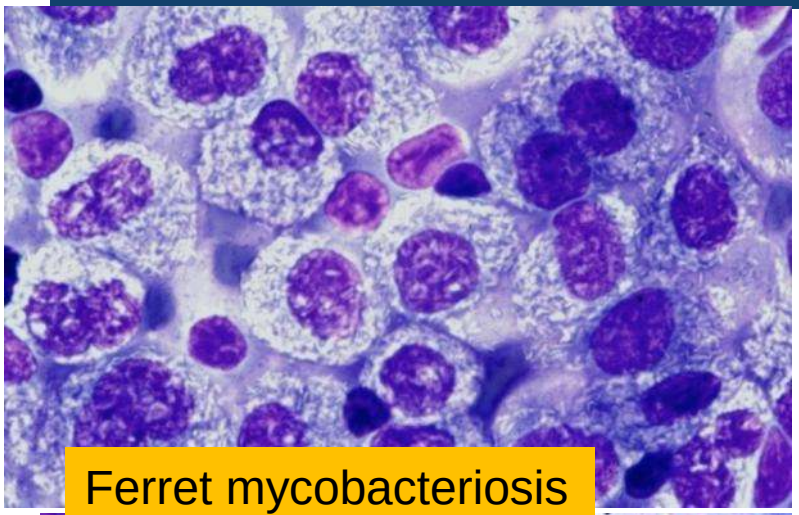


Canine fibroma and fibrosarcoma (left to right)



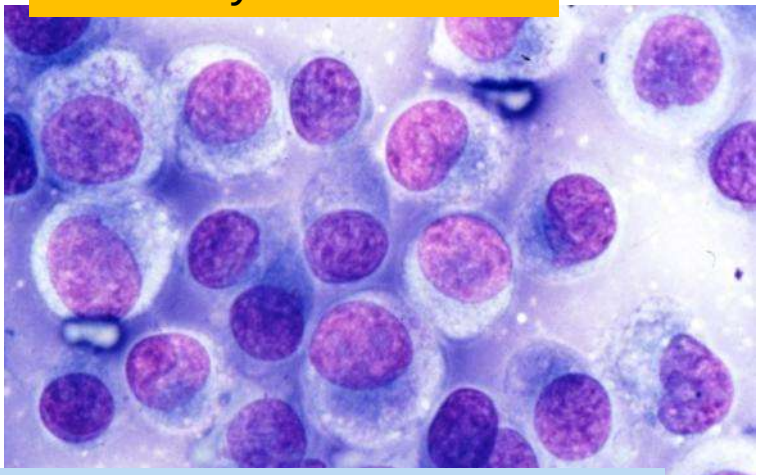
Canine malignant fibrous histiocytoma

Those round cell proliferations aren't always so easy for me! Who sometimes uses 'poorly differentiated round cell tumour' as a diagnosis?

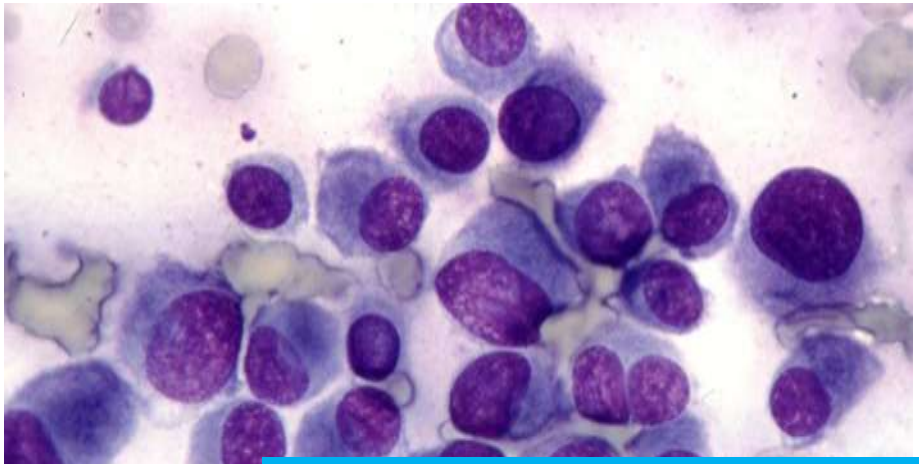


Ferret mycobacteriosis

Canine mastocytoma



Canine benign histiocytoma



Canine plasmacytoma

What do you find most difficult with skin/subcutis aspirates?

Lymph node aspirates

Key issues for me!

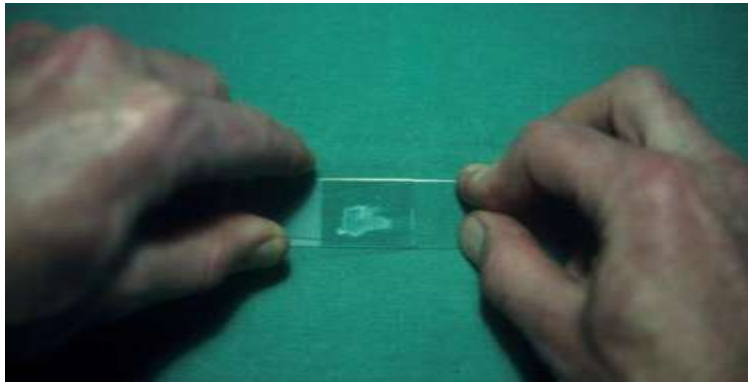
1. **Getting a representative sample**
2. **Making/having smears with minimum damage**
3. **Deciding between BLH and ML – neoplastic lymphoid cells can look like anything!**

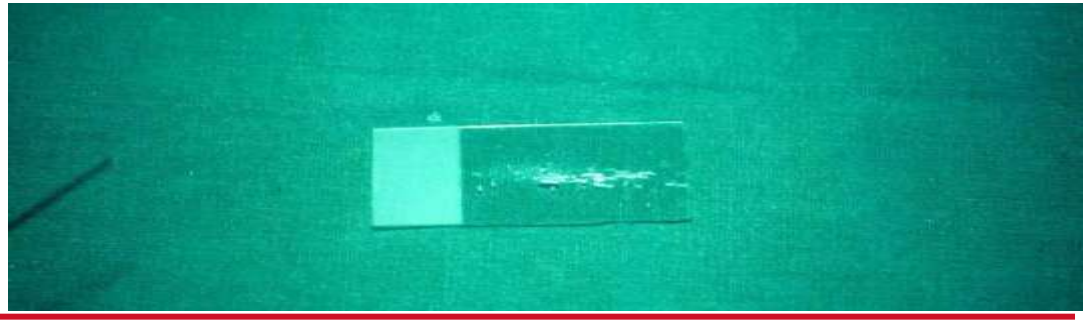
Lymphadenomegaly:

- **BLH**
- **Lymphadenitis**
- **Primary (ML) and secondary (metastatic) malignant neoplasia**

Spray technique – is it the best for lymph node aspirates?

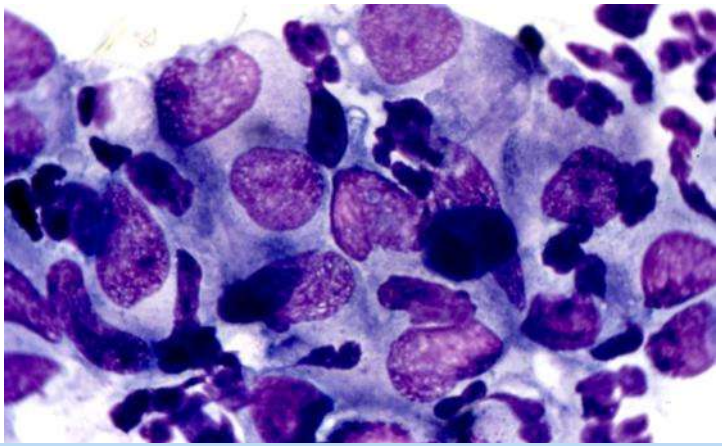
Avoids overly damaging fragile cells, but there is the problem of piling up of cells and restricted spreading of individual cells. I prefer to make the smears as for peripheral blood films. What do you prefer to make or read?



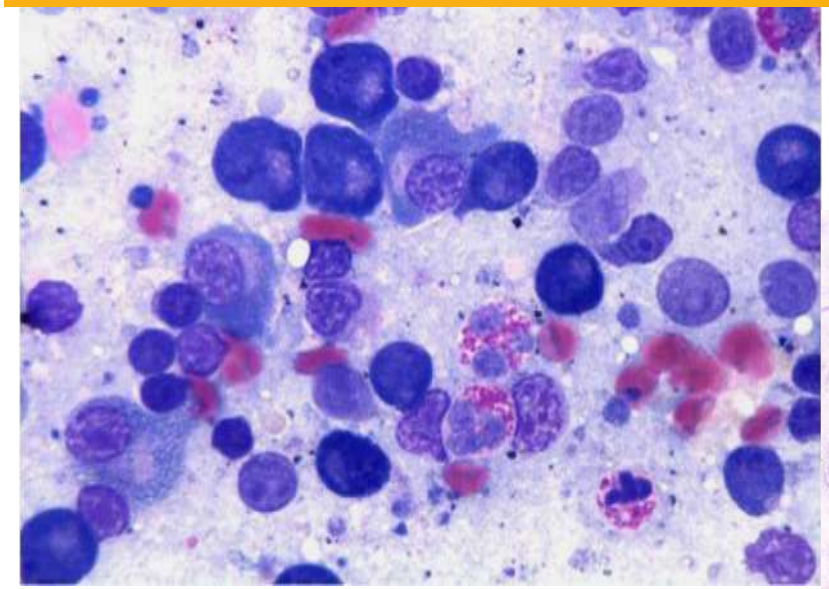


Is the lymph node really inflamed? Fortunately, lymphadenitis in the dog and cat is not common (unlike farm animals)

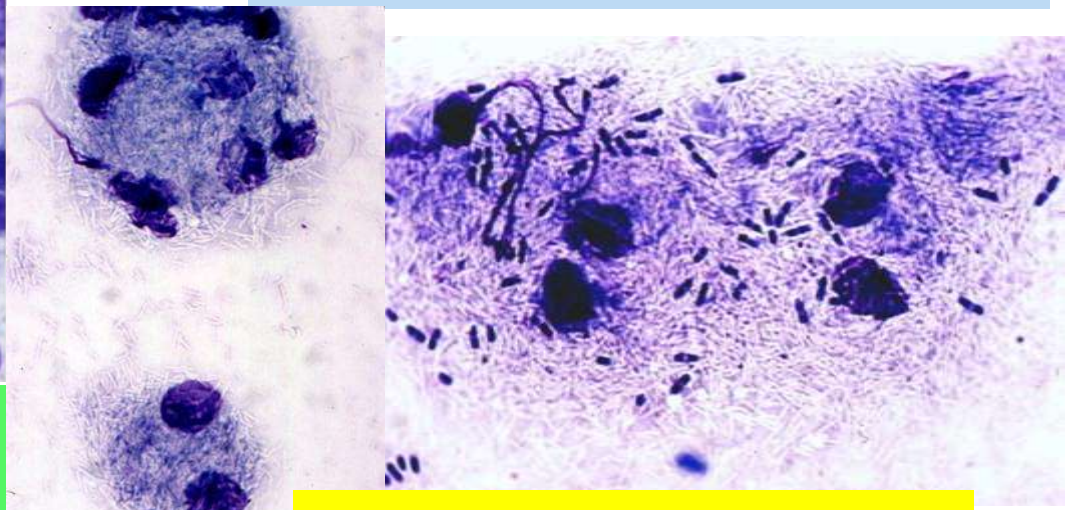
A diagnosis of lymphadenitis requires a high proportion of inflammatory cells (eg neutrophils or eosinophils >5%, but usually > 10%) OR the detection of infectious agents in the smear



Granulomatous lymphadenitis - FIP

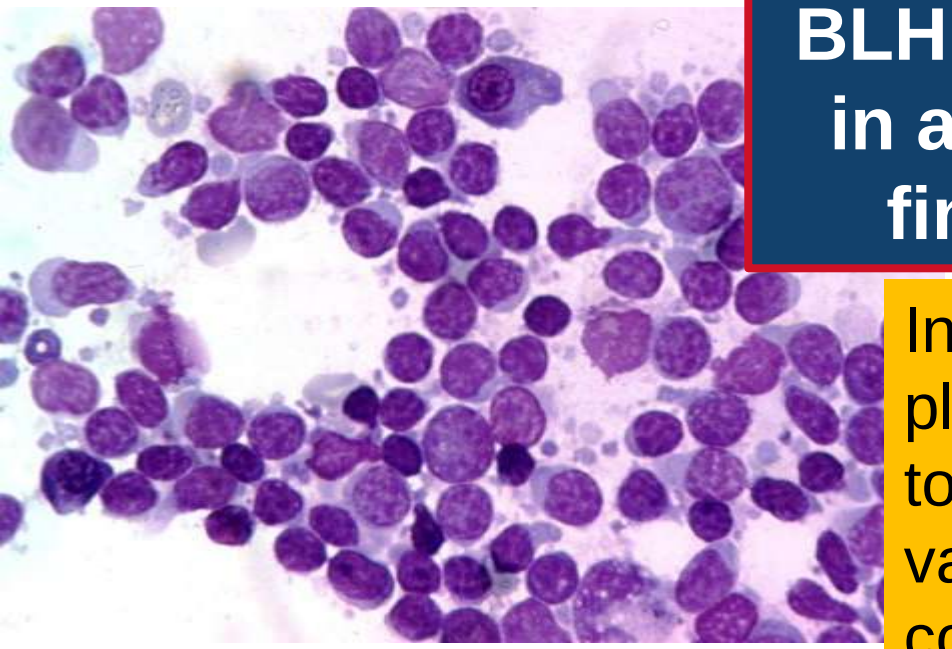


Eosinophilic lymphadenitis in a cat with EGC (9% eosinophils – true inflammation or just drainage?)

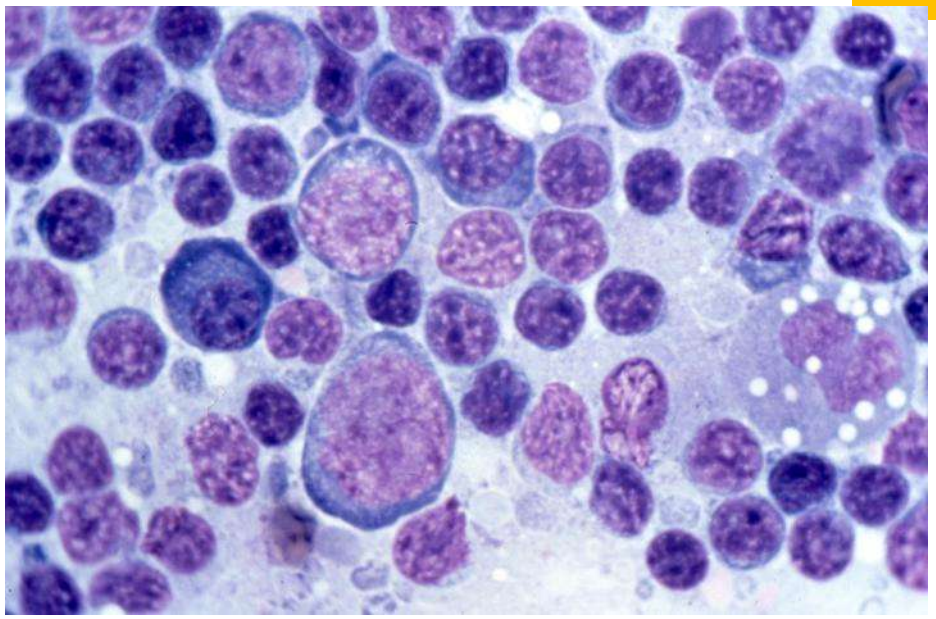


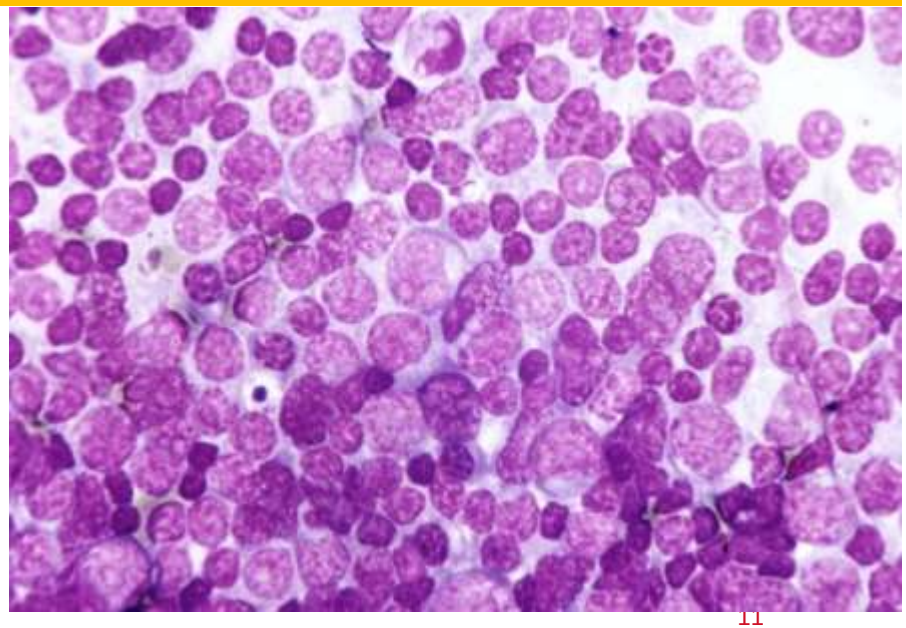
Mycobacterial lymphadenitis

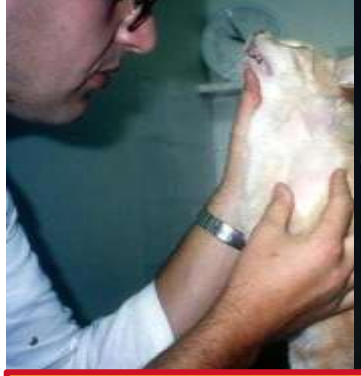
BLH - Heterogeneity is the key in any species, but I can still find it tough to be certain

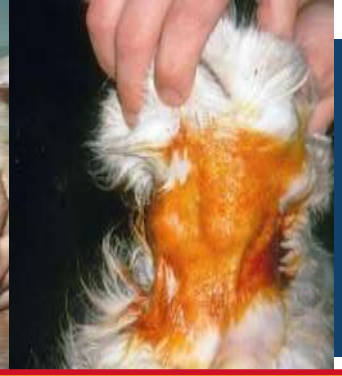


In BLH small lymphocytes and plasma cells should be >60% of total cells, but I am wary of variation between sites (and colour variation due to stain)





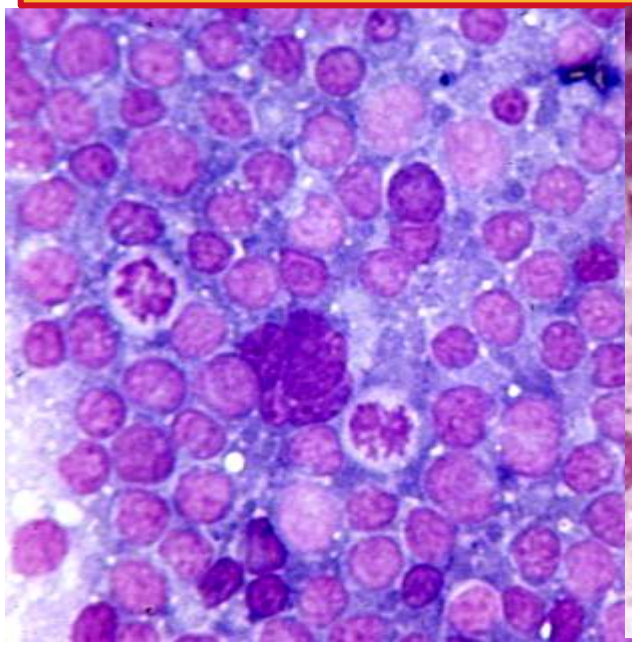


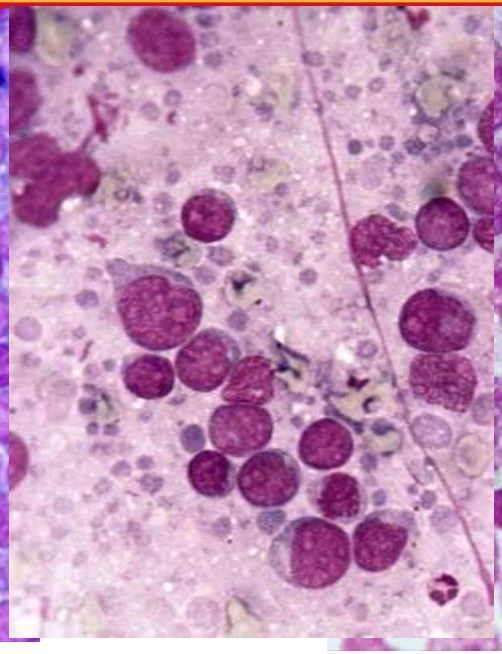


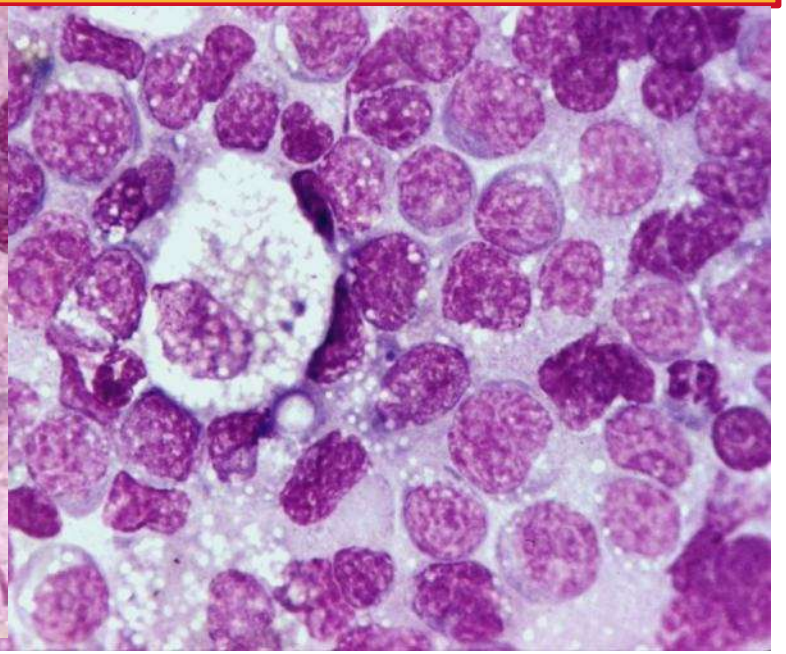
I have to remind myself that whilst lymphosarcoma is very common, it is not as common as BLH! My problem is all those variants!

Common cytological features of LN lymphosarcoma :

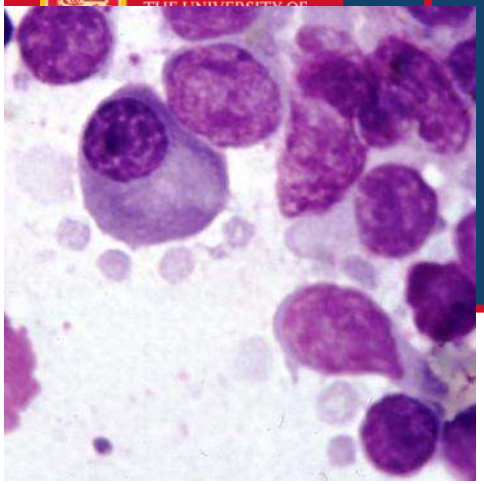
- a highly cellular sample and a feeling of homogeneity
- a high proportion of medium to large lymphoid cells (usually greater than 50-60%) – unless it is small cell variety!
- indications of high cell activity (numerous lymphoglandular bodies and tingible macrophages; increased numbers of mitotic cells) **but how specific?**

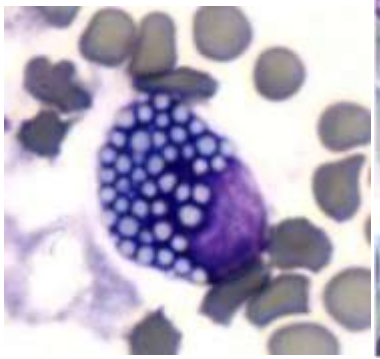


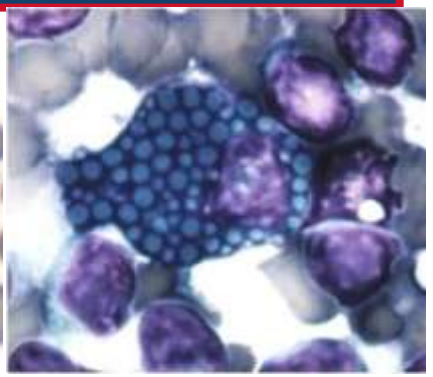


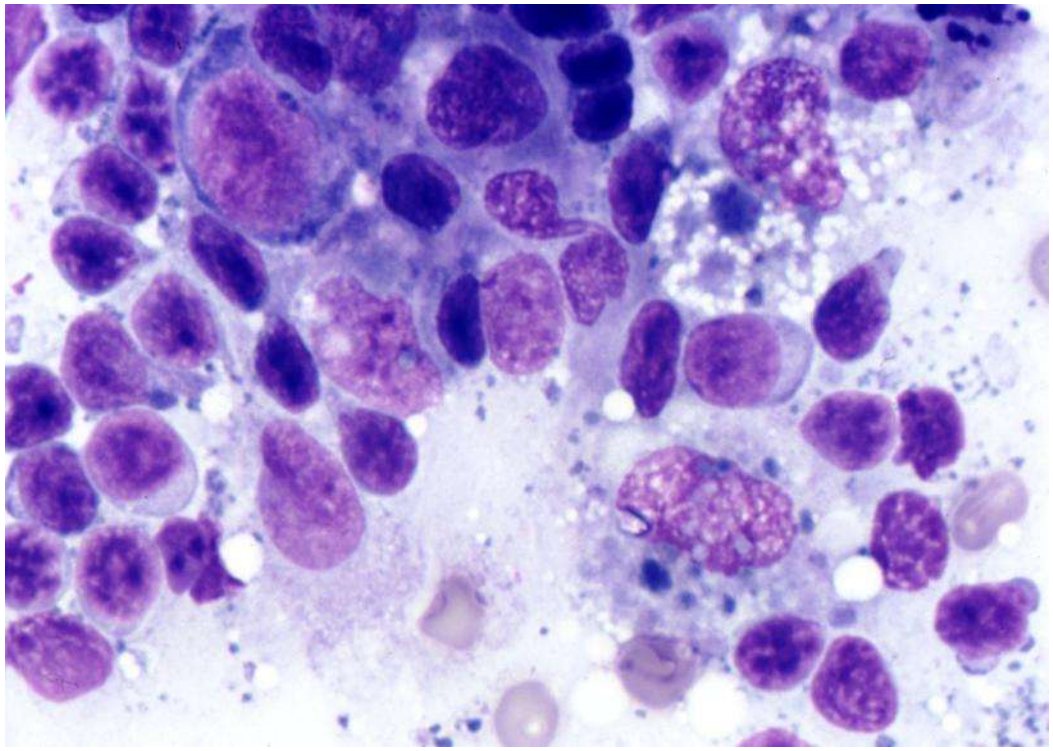


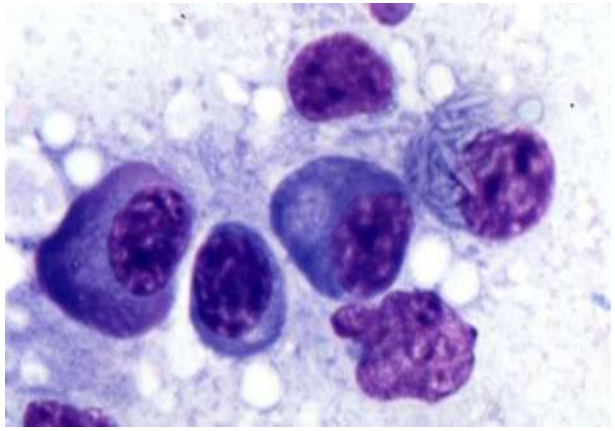
Tingible body macrophages and lymphoglandular bodies are common in both BLH and some forms of lymphosarcoma so they don't help me much!









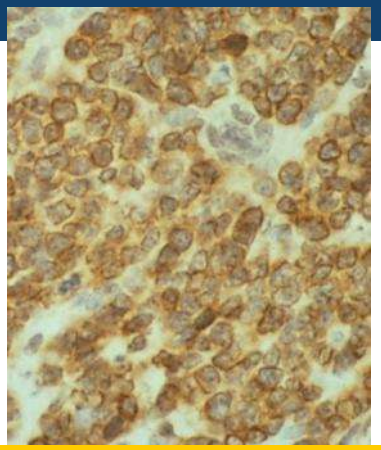


What do you think of these plasma cells?

Is immunophenotyping or cloning of use in diagnosing lymphosarcoma?

Immunophenotyping (flow cytometry or on smears through ICC) very useful because: Can confirm lymphosarcoma of T or B cell origin from other round cell tumours and -

- Canine T cell forms may have a poorer prognosis?
- Feline T cell forms may have a better prognosis?
- What about the horse and cow? All confusing!



B Cell lymphosarcoma

- Clonality Testing (mainly in dogs where called PARR [PCR for antigen receptor rearrangements]; is done in cats) - assisting in differentiating reactive lymphocytes from neoplastic ones when cytology or histopathology ambiguous?
  - Normal or hyperplastic lymphocytes should be polyclonal
  - Is it severe inflammatory bowel disease or small cell lymphosarcoma?
  - The trouble is that **some** T cell lymphosarcomas may have multiple clones (oligo to polyclonal)

I need to keep reminding myself that lymphosarcoma is a pathological process and not a cause (although it is causing all the clinical signs)!

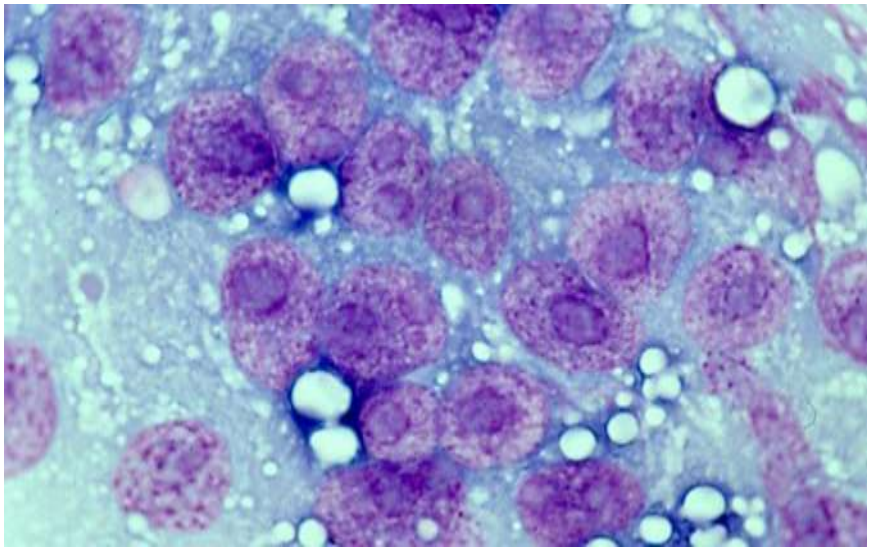
'Neoplasia is a genetic disease'? Carcinogenesis requires genetic alteration (mutation) BUT causation (aetiology) is a little more complex: for most tumours it is likely to be multifactorial

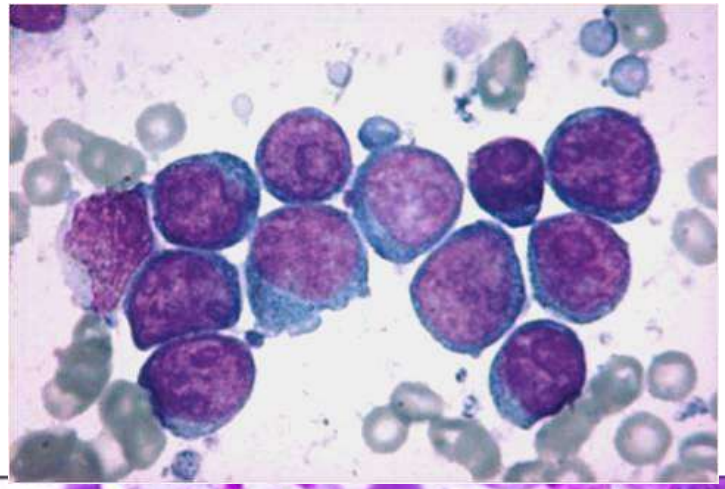
- **Physical** – none identified for lymphosarcoma? Magnetic fields?
- **Chemical** – none identified for lymphosarcoma? Pesticides in dogs?
- **Living agents** – herpesviruses and oncoviruses!
- **Genetic (heritable –** *does it need a trigger?***)** – Boxer?
- **Immune-mediated** – suppression of immune surveillance. FIV? KIV? Genetic?

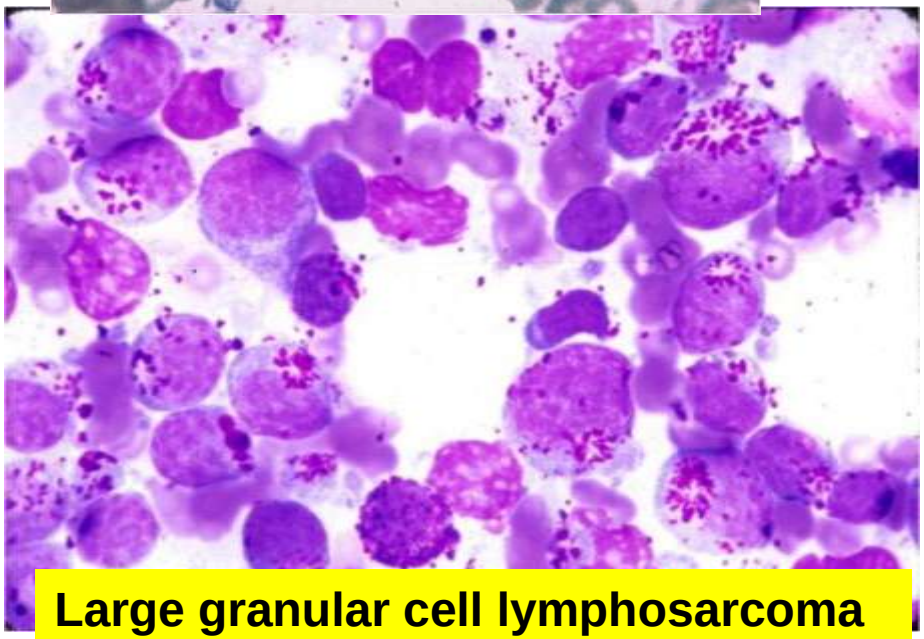
I keep getting caught about not remembering all those paraneoplastic effects of neoplasms on clin path results!

- Mostly due to chemical release or due to expression of antigens that are viewed as ‘foreign’ by the remaining functional immune system
- Lymphosarcoma – many reported
  - ‘Autoimmunity’ important – mechanisms poorly understood, possibly more than one cell type involved (IMHA most common)
  - Dysproteinaemias (mono- or polyclonal gammopathies), cytokines (eg IL-5 and eosinophils), hormonally active chemicals (eg hypercalcaemia)

Neoplastic cells can look like anything – but you are looking for homogeneity (or is it monotony)!



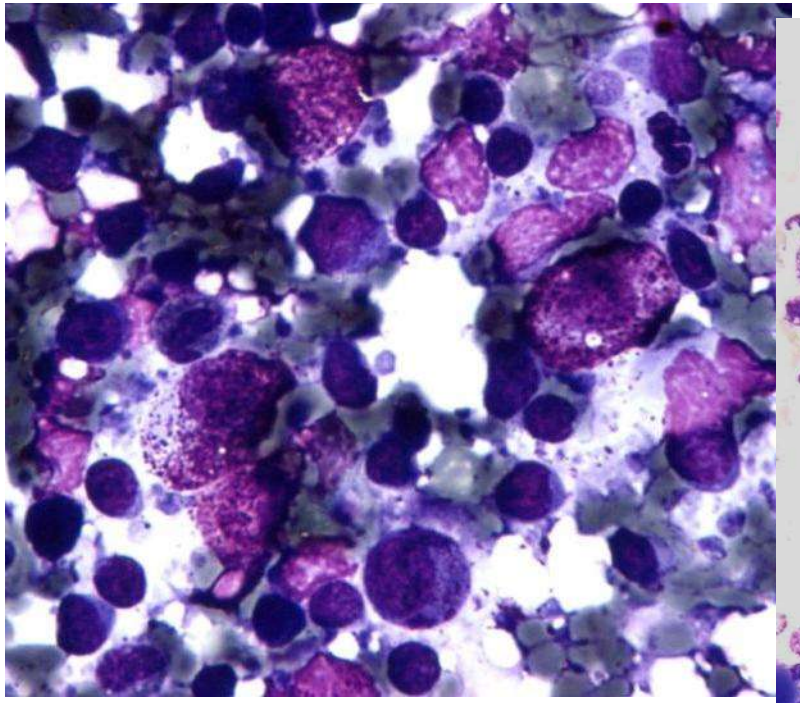




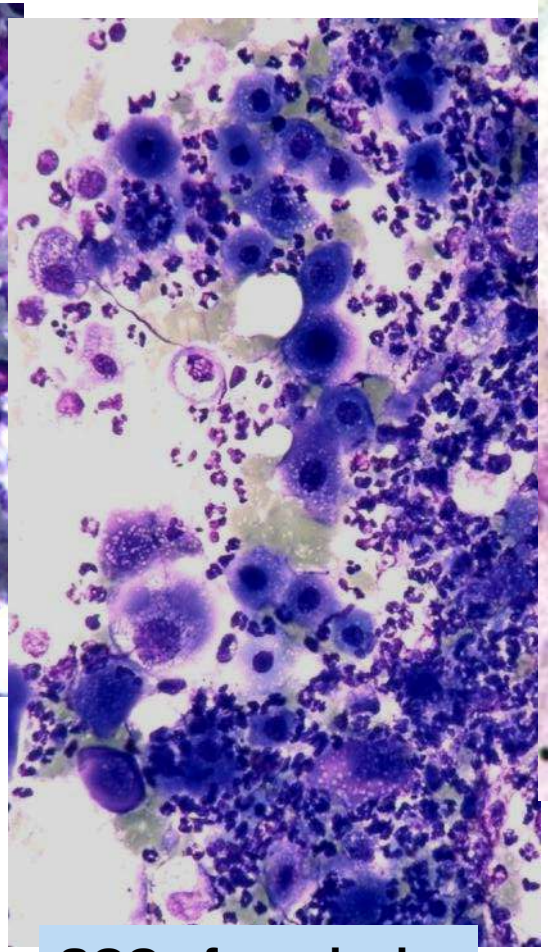
Large granular cell lymphosarcoma

It is easier to damage neoplastic lymphoid cells than small lymphocytes. I need to remember to take this into account when doing the percentages!

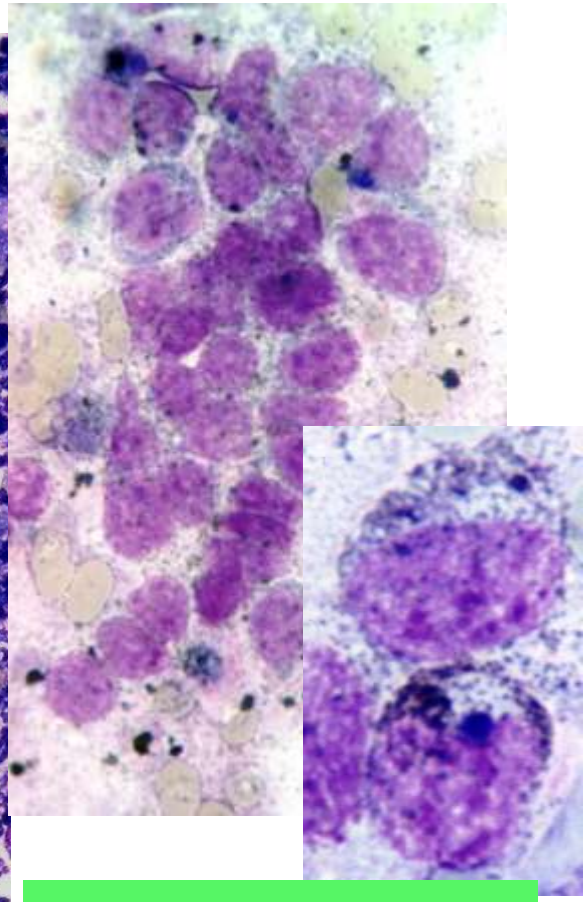
Metastatic neoplasia affecting lymph nodes is common – but it may or may not cause lymphadenomegaly. Unless diffusely involved, it is easy to miss with an FNA!



Mast cells – just draining or true metastasis? Those round cells are a problem for me!



SCC of cervical lymph node



Malignant melanoma (melanosarcoma) of cervical node

Seven years old male crossbred dog with a prolonged history of polyuria, polydipsia. The dog had pale mucous membranes, lethargy and inappetence. Splenomegaly and hepatomegaly were detected on examination.

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	**0.20**	0.37-0.50
Plasma protein g/L	60	55-75
Haemoglobin g/L	**68**	100-150
Erythrocytes $x10^{12}$/L	**3.1**	5-7
MCV fl	64	60-75
MCHC g/L	340	300-360
Leukocytes $x10^9$/L	**5.8**	7-12
Neutrophils (seg.) $x10^9$/L	**2.0**	4.1-9.4
Neutrophils (band) $x10^9$/L	**0.8**	0-0.24
Lymphocytes $x10^9$/L	2.7	0.9-3.6
Monocytes $x10^9$/L	0.3	0.2-1.0
Eosinophils $x10^9$/L	**0**	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film: **1 NRBC per 100 leukocytes, most of the lymphoid cells are large**		
Platelets $x10^9$/L	390	200-900
Reticulocyte % (uncorrected)	0.2	0-1.5

Haematology revealed:

- Non-regen anaemia
- Leukopenia due to neutropenia (left shift – is this what they used to call 'degenerative'?)
- Absolute eosinopenia
- Large lymphoid cells – reactive or neoplastic?

More Clin Path

BIOCHEMISTRY	SAMPLE	REFERENCE INTERVAL
ALP IU/L	80	<110
ALT IU/L	55	<60
Serum protein (biuret) g/L	58	50-70
Albumin (BCG) g/L	30	23-43
Globulins g/L	28	27-44
Glucose mmol/L	5.0	3.3-6.4
Urea mmol/L	**34**	3-10
Calcium mmol/L	**3.1**	2.1-2.9
Inorganic phosphate mmol/L	**4.2**	0.8-1.6

Biochemistry revealed:

- azotaemia
- Hypercalcaemia (corrected = 3.17)
- Hyperphosphataemia
- Urine SpG?

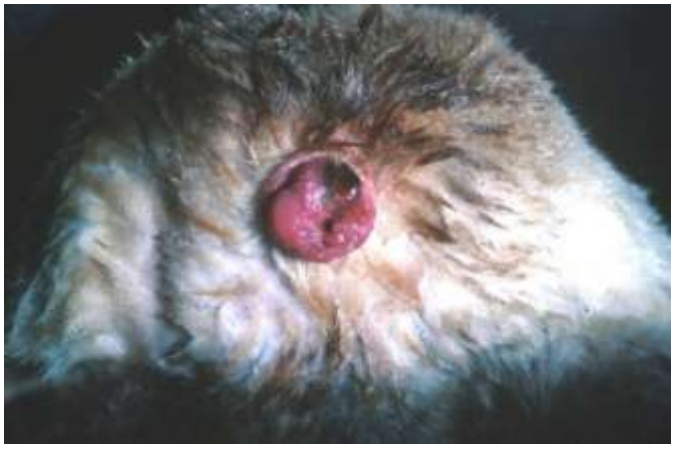
URINALYSIS (voided)			
Appearance	Clear	pH	6.0
Colour	Light yellow	Glucose	-ve
Specific gravity	1.019	Ketones	-ve
Protein	-ve	Blood	-ve
		Bilirubin	Trace
Microscopic findings: little seen			

DIAGNOSIS of **pseudohyperparathyroidism and hypercalcemic nephropathy associated with lymphosarcoma and lymphoid leukemia** was confirmed at necropsy and subsequent histopathology. Abdominal lymph nodes were also affected.

Hypercalcemia can occur in cases of lymphoid neoplasia, and occasionally in multiple myeloma (*paraneoplastic syndrome*). It is thought to be primarily due to the production of substances that enhance bone resorption (parathormone-like protein, prostaglandins, Vitamin D-like steroid and osteoclast activating factor - hence the name **pseudohyperparathyroidsm**). Other tumours can produce similar substances in the dog such as apocrine gland adenocarcinoma of the anal sacs. Commonly, inorganic phosphate is not elevated in cases of pseudohyperparathyroidism unless there is some interference in renal outflow. This appears to be the case in this dog and is probably due to the deposition of calcium in the kidney interstitium and tubules. Some tumours cause hypercalcemia by metastasising to the bones and causing osteolysis. This may be by direct action or by activating osteoclasts. It is still referred to as pseudohyperparathyroidism. In these situations the inorganic phosphate may be elevated.

Yes, I had to use a koala! Lymphosarcoma is the most common neoplasm in just about every species. Is that because the immune system started off its life as tumour cells that were 'tamed' (heretical)?

- 7 years old female koala ('Doreen') presented for 'wet bottom'. In poor condition and cried on urination.
- **On examination:** also had enlarged inguinal lymph nodes as well as swollen 'cloacal' mucosa



Haematology

TEST	SAMPLE	REFERENCE RANGE
Plasma appearance	clear	Clear
PCV l/l	**0.18**	0.29-0.44
Plasma protein g/l	**53**	60-83
Leukocytes $x10^9$/l	**15.9**	2.8-11.2
Neutrophils (seg.) $x10^9$/l	**2.8**	0.5-6.3
Lymphocytes $x10^9$/l	**11.6**	0.2-5.8
Monocytes $x10^9$/l	**1.0**	0.0-0.6
Eosinophils $x10^9$/l	0.5	0.0-1.1
Basophils $x10^9$/l	0	0-0.12
Platelets $x10^9$/l	**150**	200-400
Reticulocyte % (uncorrected)	0.4	0-1.0
Blood film: **60 nucleated erythroid cells per 100 leukocytes counted. Many lymphoid cells are medium to large and have prominent nucleoli**		

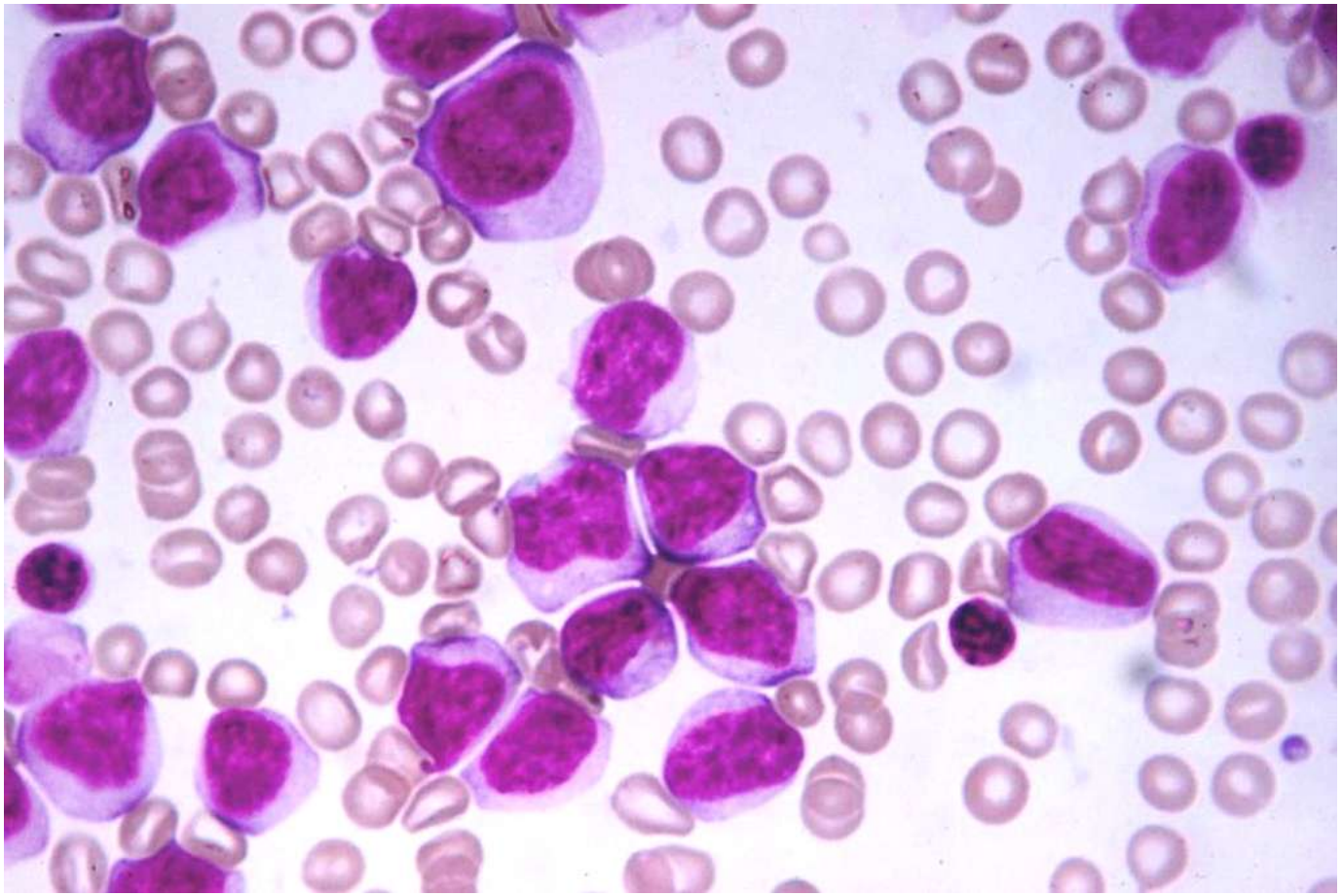
- Non-regen anaemia
- hypoproteinaemia
- Leukocytosis due to lymphocytosis and monocytosis
- Thrombocytopenia
- Circulating cells?

Leukaemia?

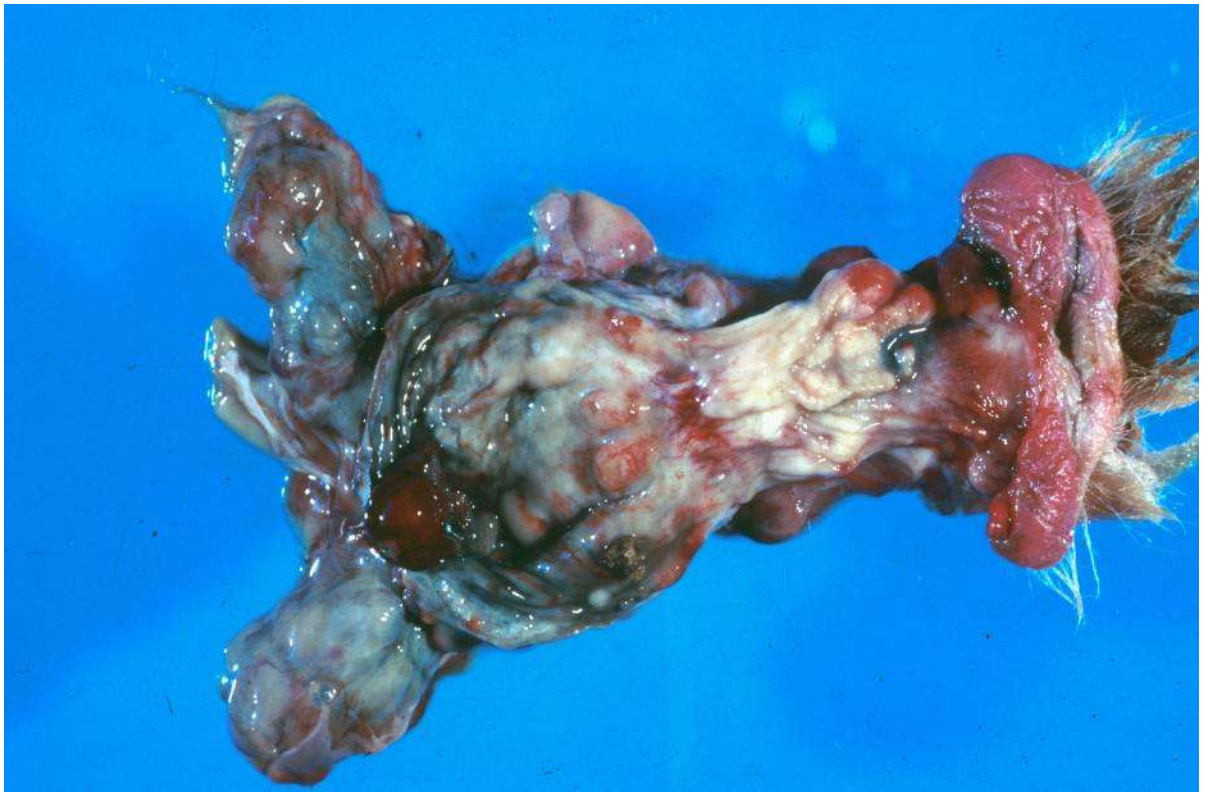
Anaemia, hypoproteinaemia and monocytosis related to chronic disease?

Why the thrombocytopenia?

Lymphoid leukaemia – secondary to lymphosarcoma?



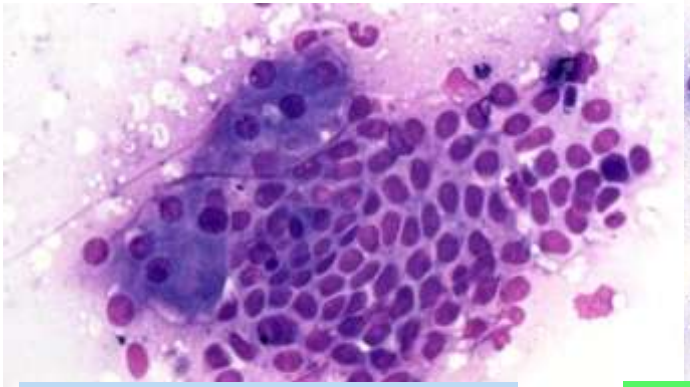
What do you think was the outcome for the koala?



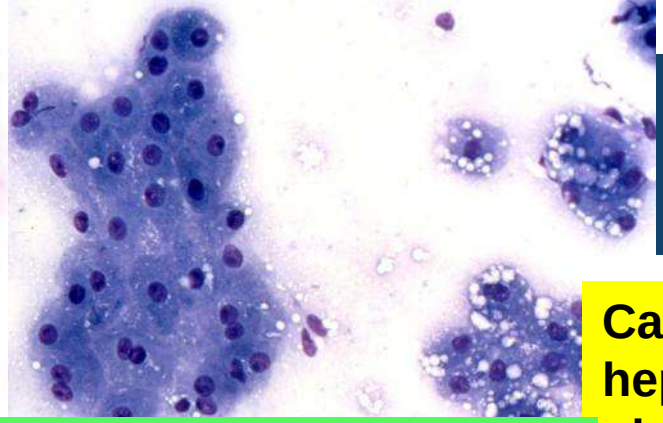
Liver – correlation between histo and cyto has been reported as low as 30% and as high as 70% (full agreement). Why?

› What are the difficult parts for me?

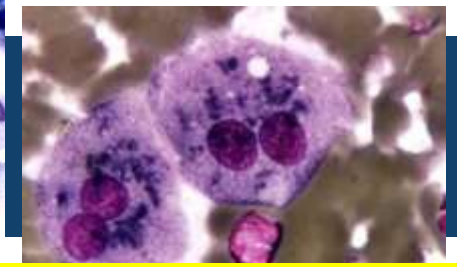
- **Getting a decent sample!** Mostly US guided FNA's, and can be hit or miss (especially if nodular or multifocal). Scrapings and imprints of liver tissue far more correlative with histopathology (surprised?)
- **Degenerative issues?** Sometimes the changes are subtle. Sometimes you are not sure if it is a normal variation for hepatocytes
- **Inflammatory issues** (distinguishing between EMH and cholangitis – and myeloid leukaemia!)
- **Neoplastic issues**: differentiating hyperplasia, benign neoplasia and malignant neoplasia is a problem for me!
  - The dilemma of secondary infection and necrosis in neoplasia. You can miss the tumour!



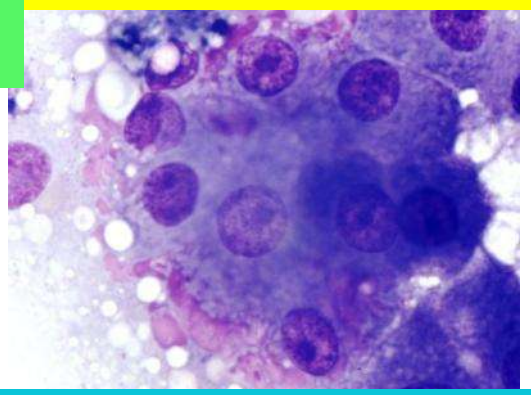
Canine hepatocytes adjacent to biliary epithelium



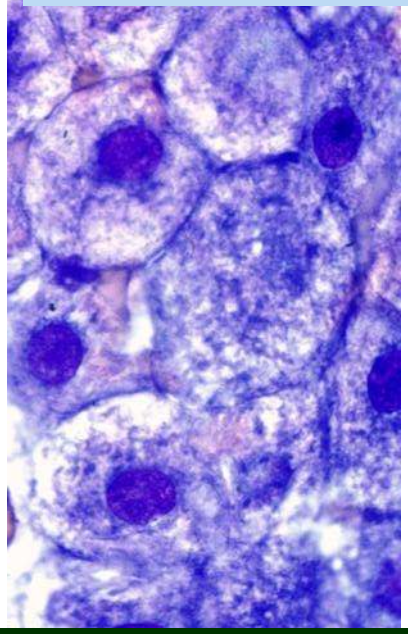
Aged Canine hepatocytes with mild vacuolar change

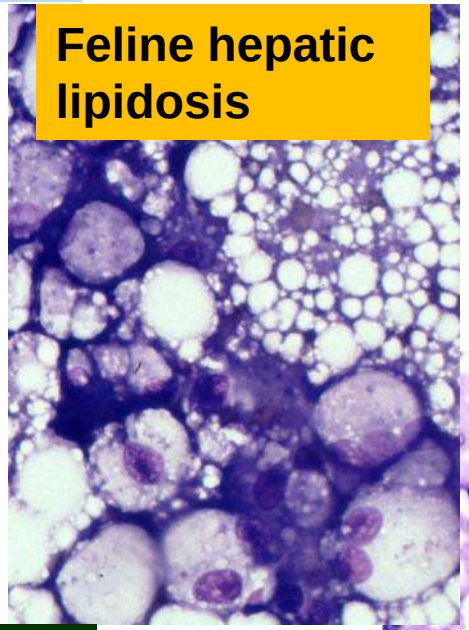


Canine binucleate hepatocytes containing pigment – normal?



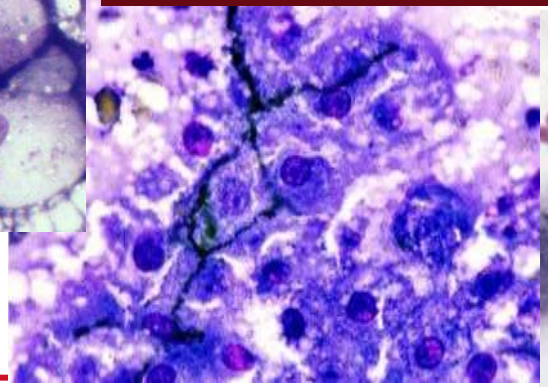
Hepatic amyloidosis in a cat

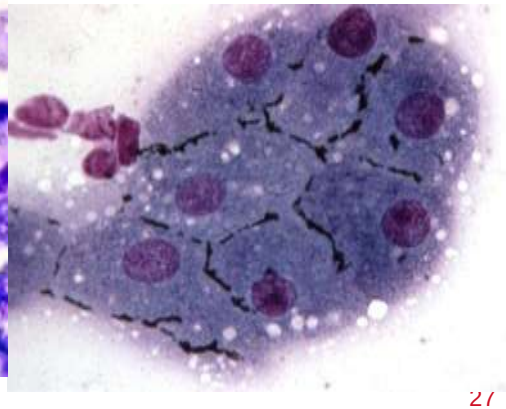




Feline hepatic lipidosis

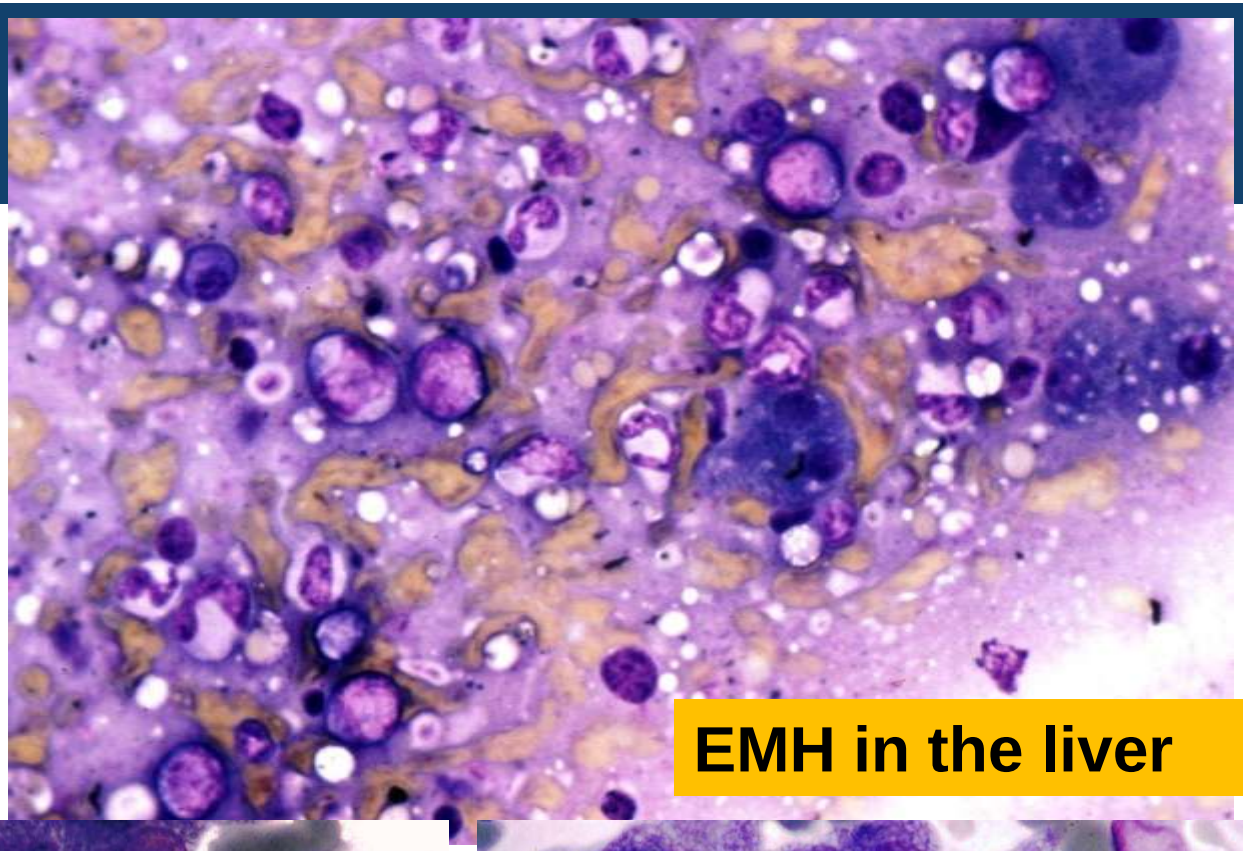
Canine hepatocytes and cholestasis



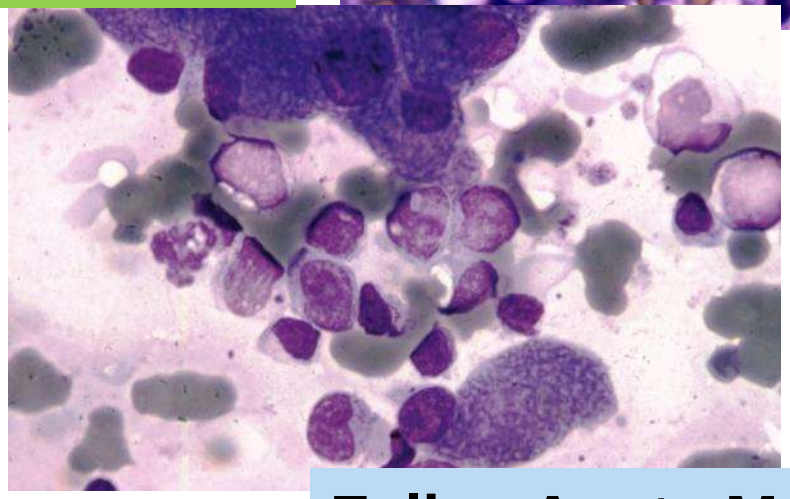


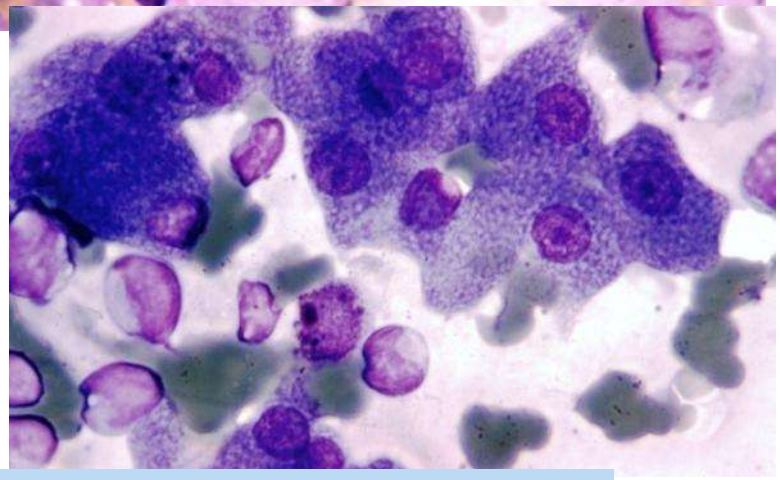
Canine hepatocytes with glycogen deposition – HyperA)

Feline EMH and AML – which is which?

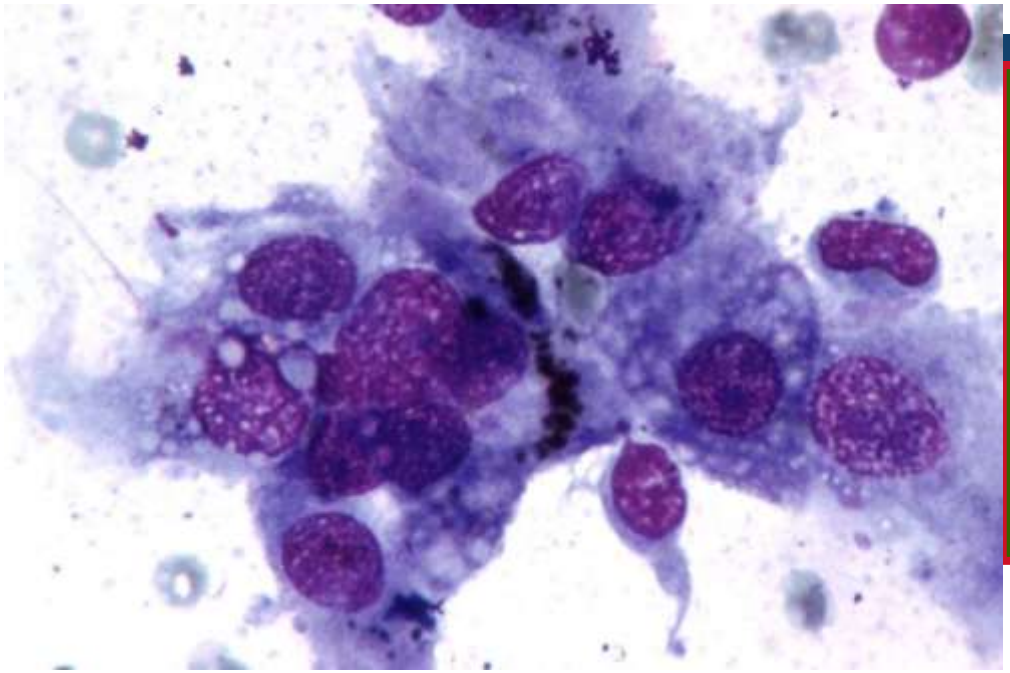


EMH in the liver





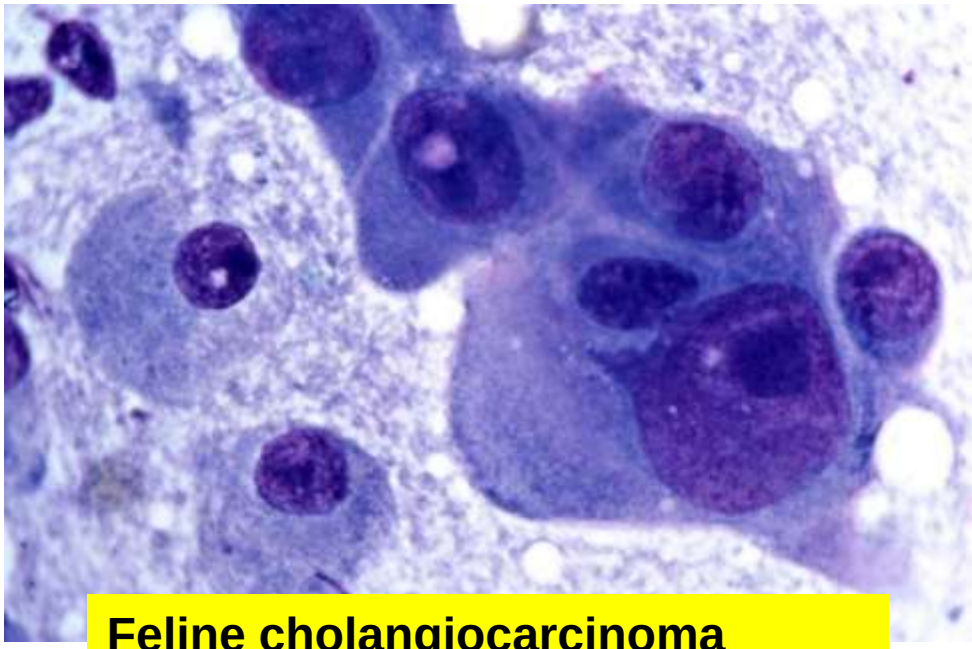
Feline Acute Myeloid Leukaemia



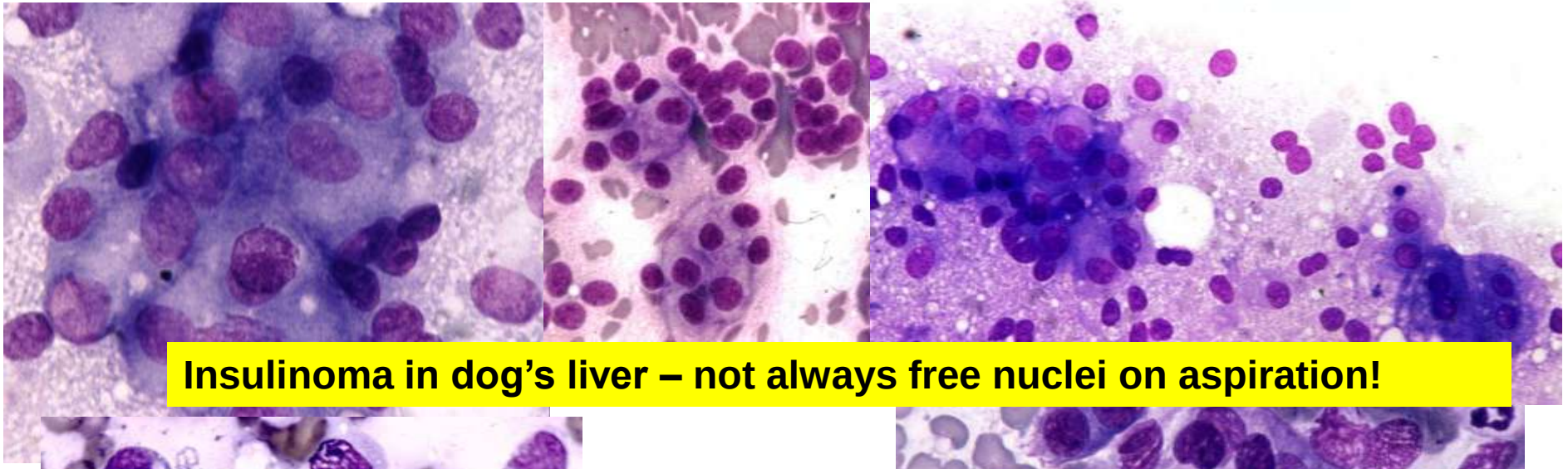
Hyperplasia/adenoma/ well differentiated carcinoma in the dog? A big dilemma for me. Is it shared with anatomical pathologists?

Canine hepatic carcinoma

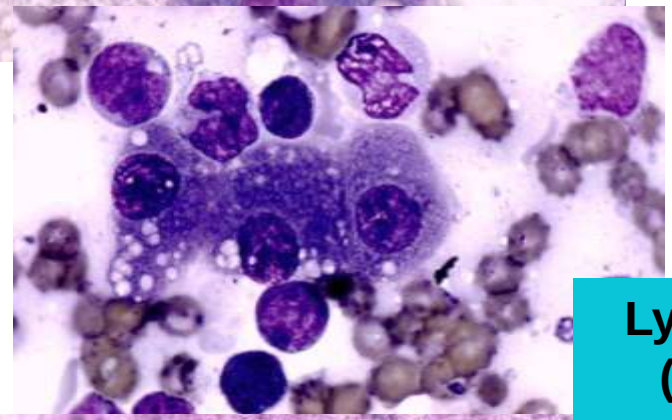
These two were relatively easy!

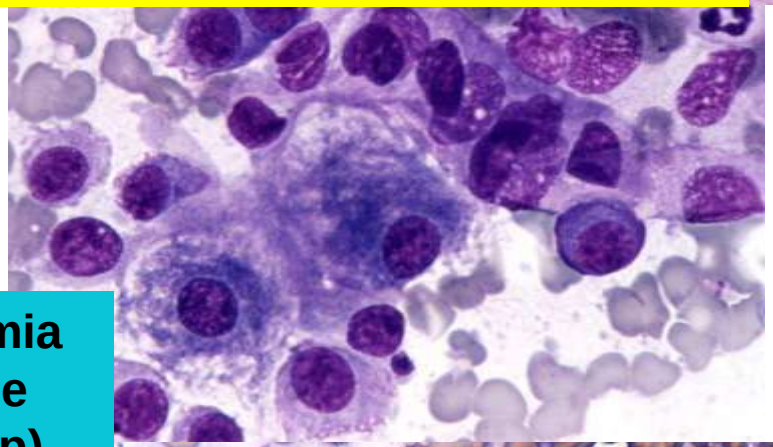


Feline cholangiocarcinoma

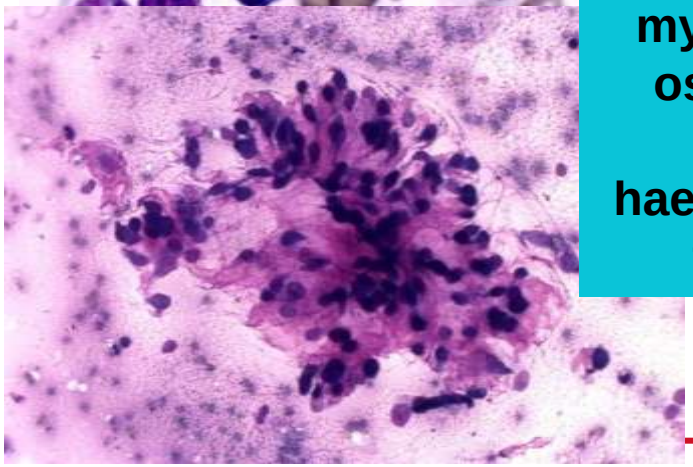


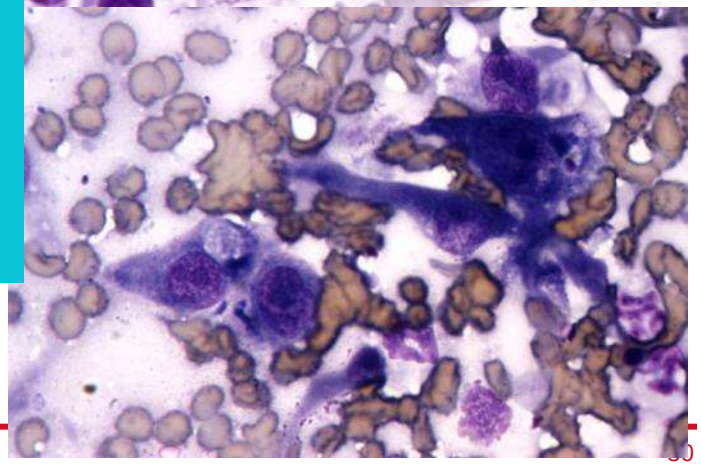
Insulinoma in dog's liver – not always free nuclei on aspiration!



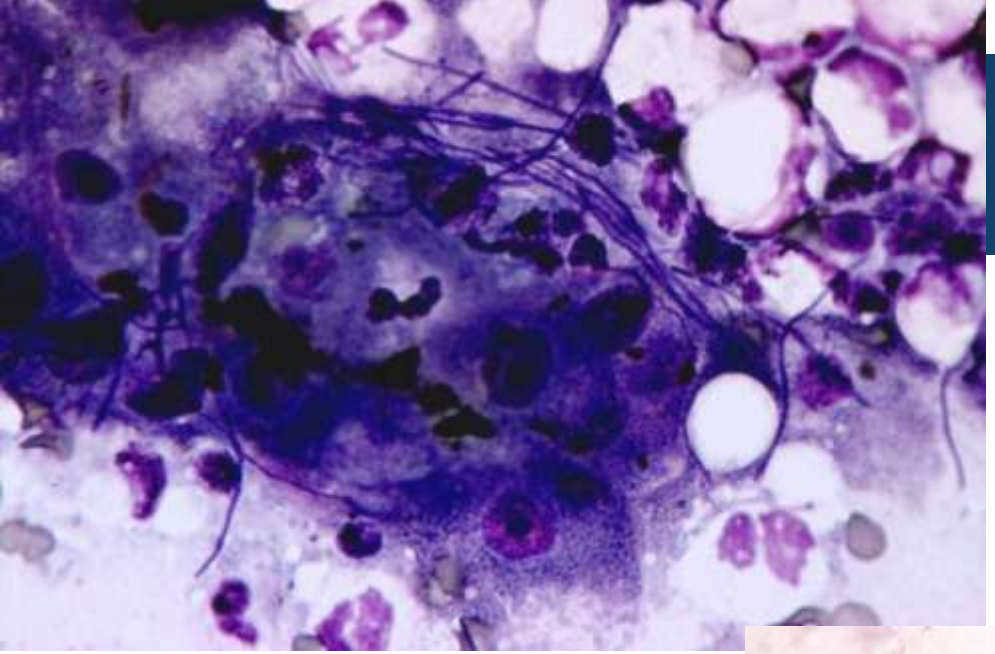


Lymphoid leukaemia (left top), multiple myeloma (right top), osteosarcoma (left bottom) and haemangiosarcoma in the canine liver



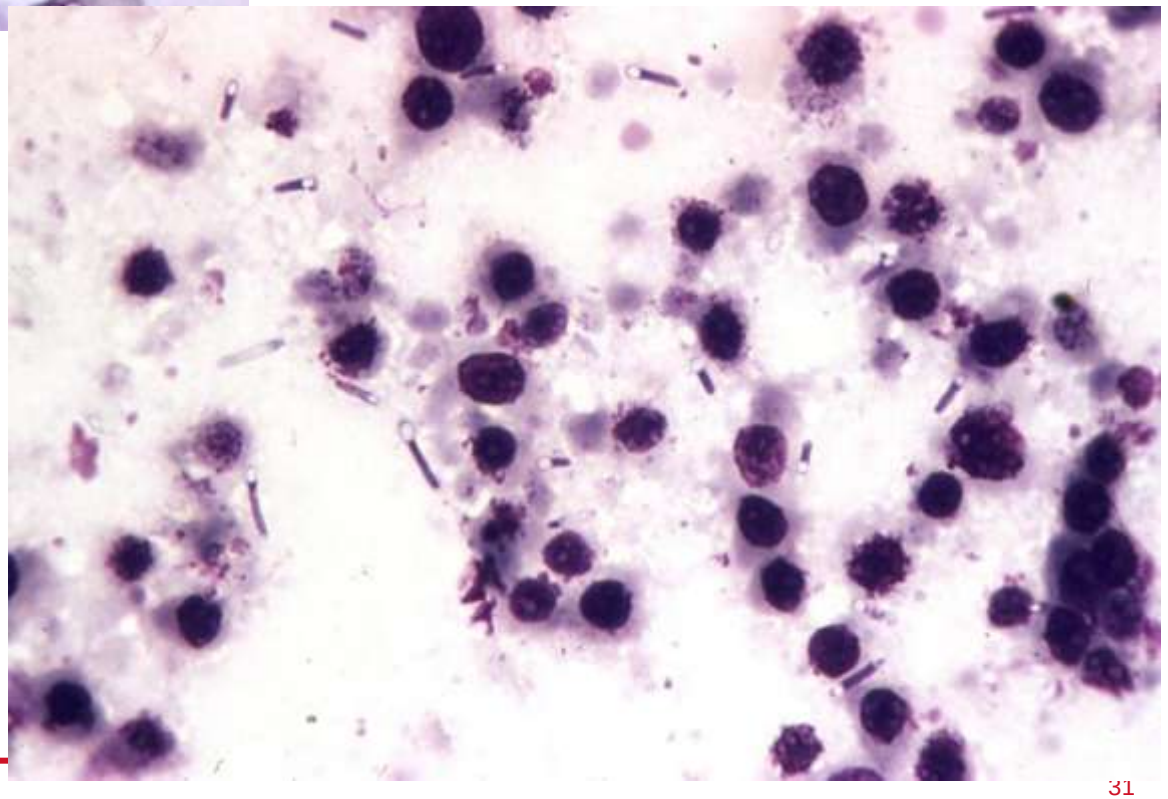


Complication of hepatic neoplasia



Feline hepatic carcinoma and clostridial hepatitis

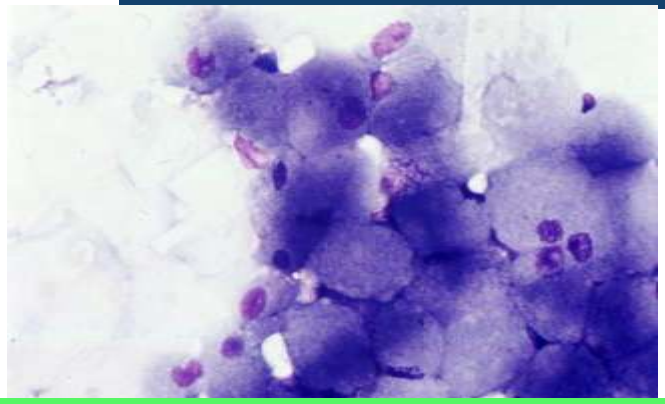
Canine well differentiated hepatic carcinoma (necrotic) and clostridial hepatitis



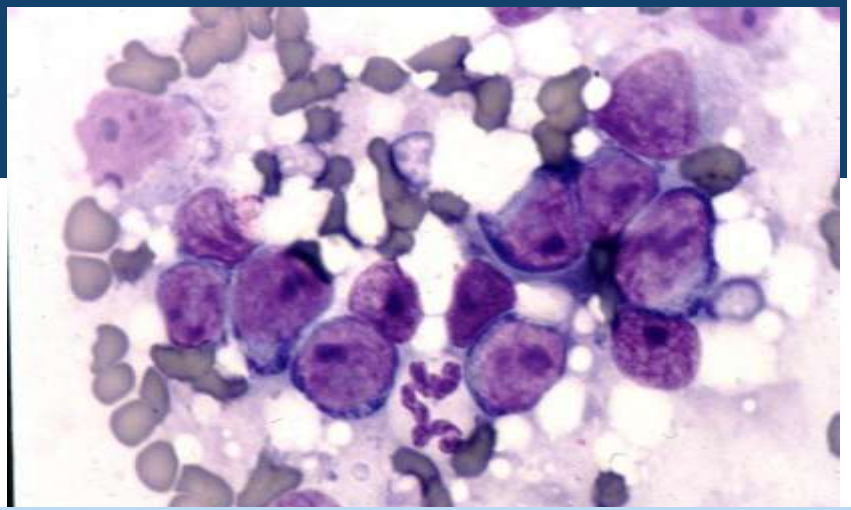
Kidney: What are the difficult parts for me?

- Getting a decent sample!
- Degenerative issues?
- Those lymphocytes! Neoplastic or inflammatory?
- Neoplastic issues: types of carcinomas (renal, collecting duct, TCC). Can we really differentiate them?

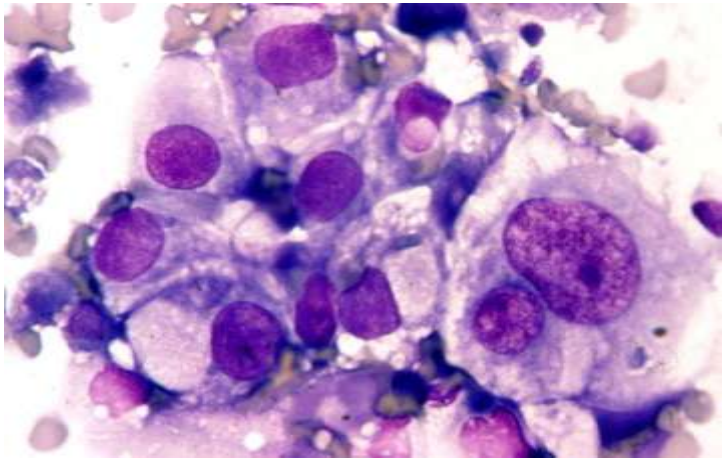
Kidney



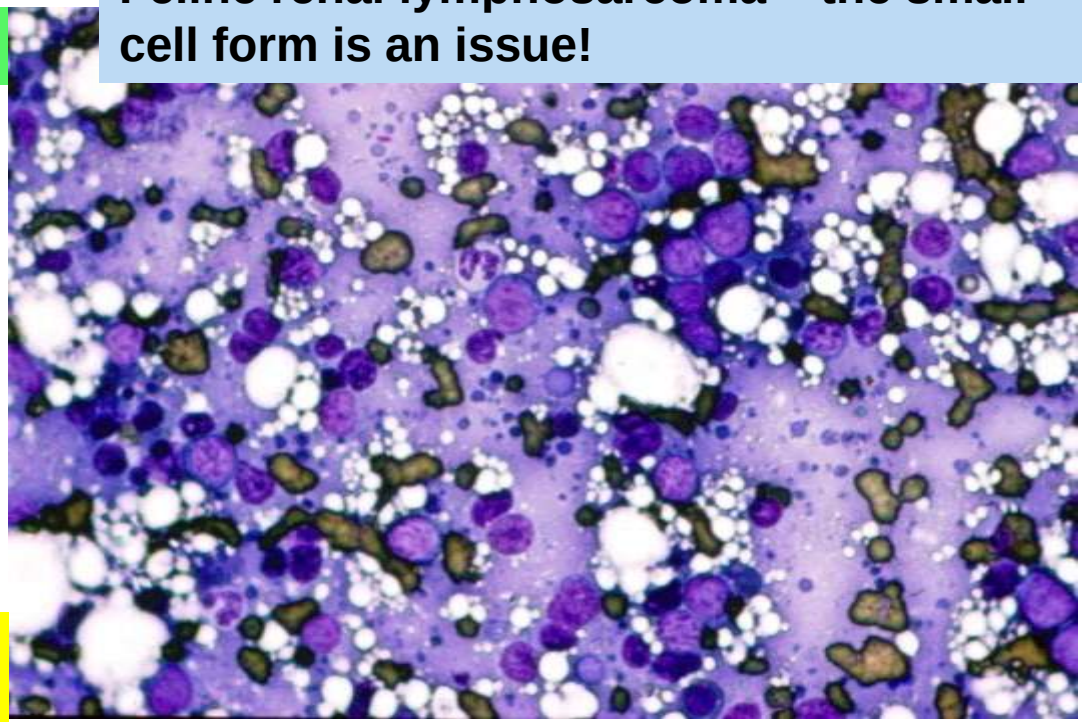
Canine perirenal cysts



Feline renal lymphosarcoma – the small cell form is an issue!



Transitional cell (?) carcinoma – renal pelvis of a cat

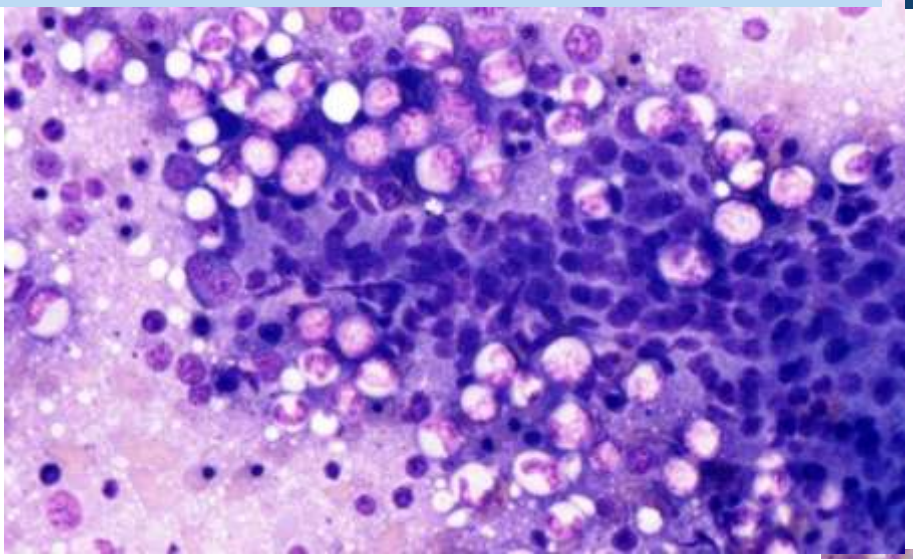


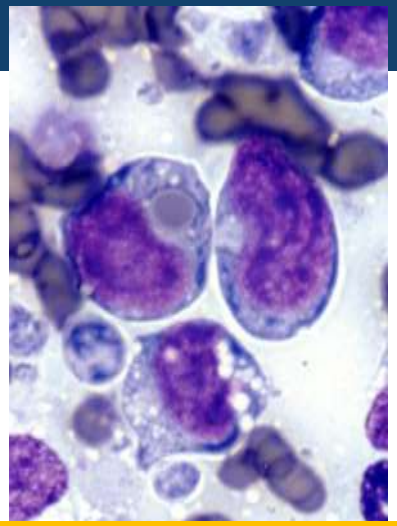
Splenic aspirates always bloody!

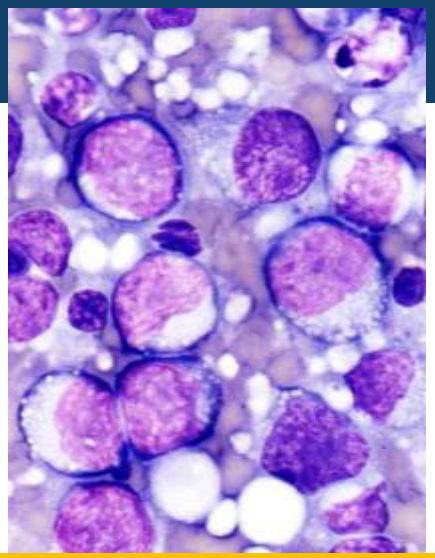
- One canine study (Balegeer *et al* 2007 JAVMA) suggested 60% correlation between histopathology and cytopathology, but my experience (canine) has been better (59% complete agreement; 29% partial agreement)!
  - I find that it is best to group benign hyperplasia, haemorrhage/haematoma, and necrosis . Any of these may accompany inflammation or malignancies
  - I find that FNA's (ultrasound-guided of course) are good for malignant neoplasia, but not as good for diagnosing splenitis

Splenic Aspirates

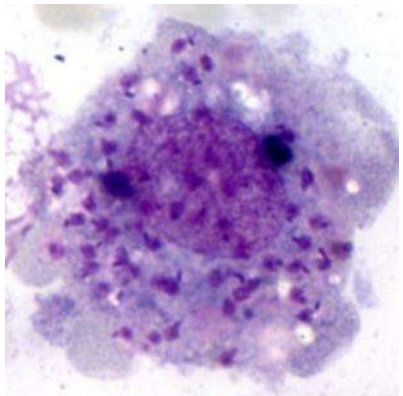
Benign splenic hyperplasia in a Labrador



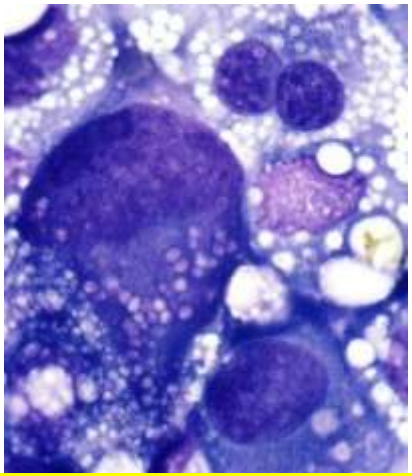




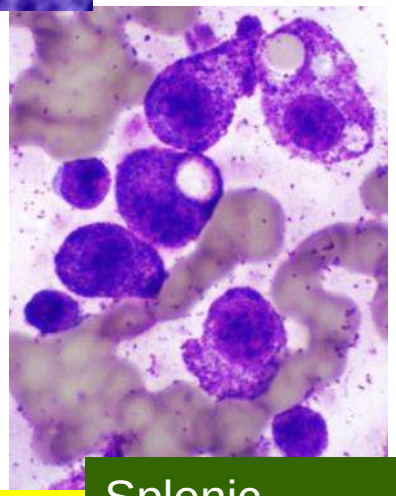
Splenic lymphosarcoma in a cat and dog



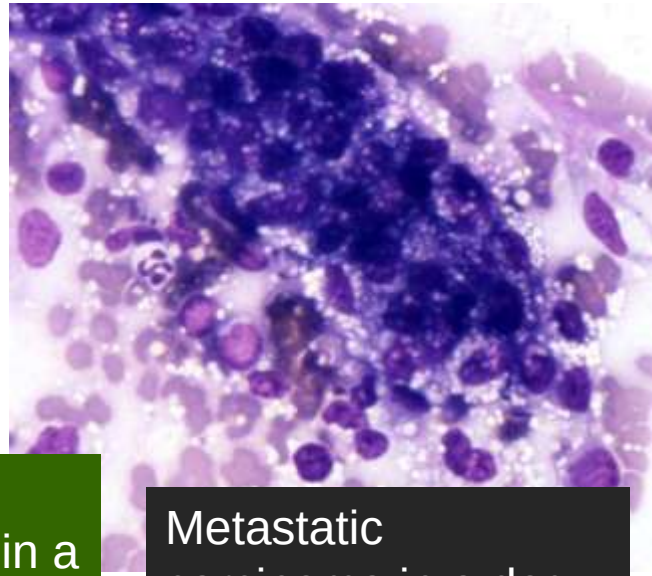
Leishmaniasis in a Chow dog from Malta



Malignant histiocytosis in a Rottweiler



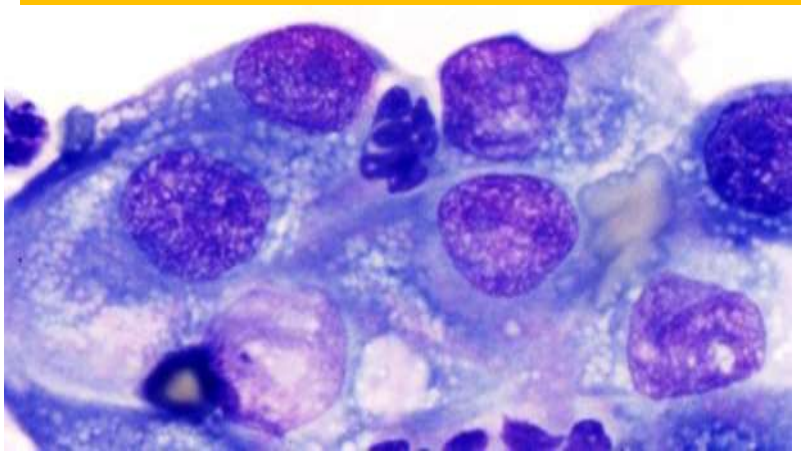
Splenic mastocytosis in a cat

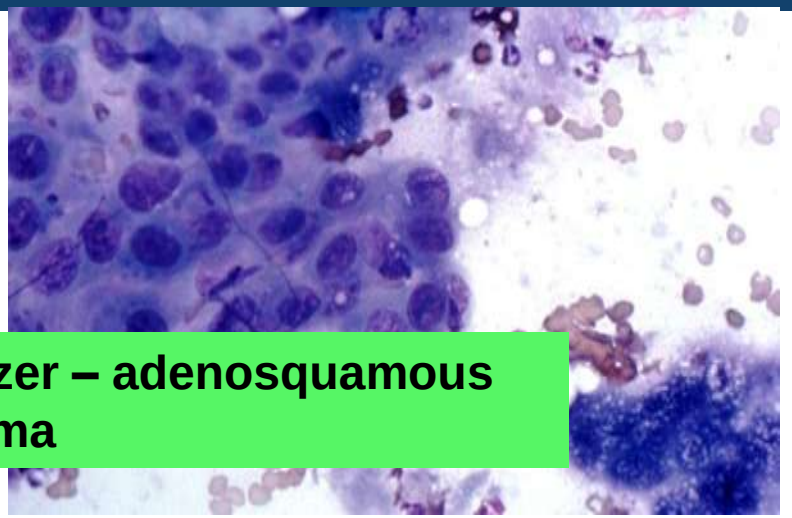


Metastatic carcinoma in a dog

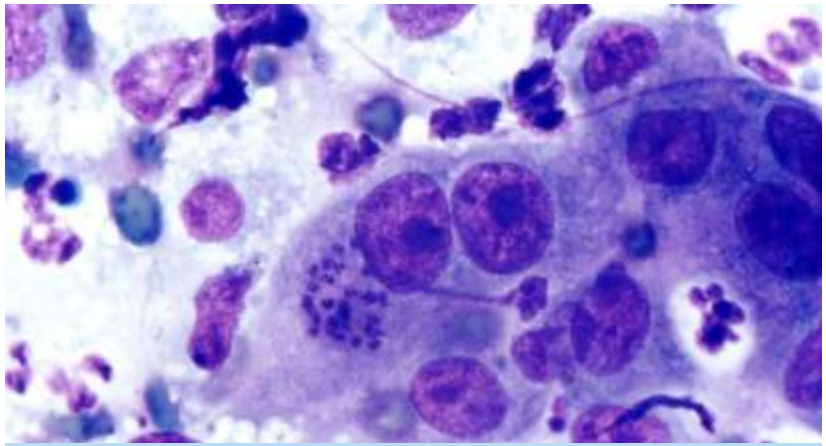
Lung FNA's- Histopathology correlative studies – 80-90% (mainly nodular lesions)

Feline pulmonary adenocarcinoma





Schnauzer – adenosquamous carcinoma



Burmese cat - adenomatosis and toxoplasmosis

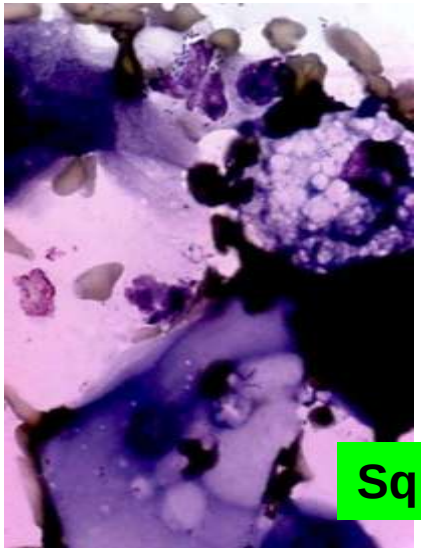
Haemangiosarcoma of the lung in a dog

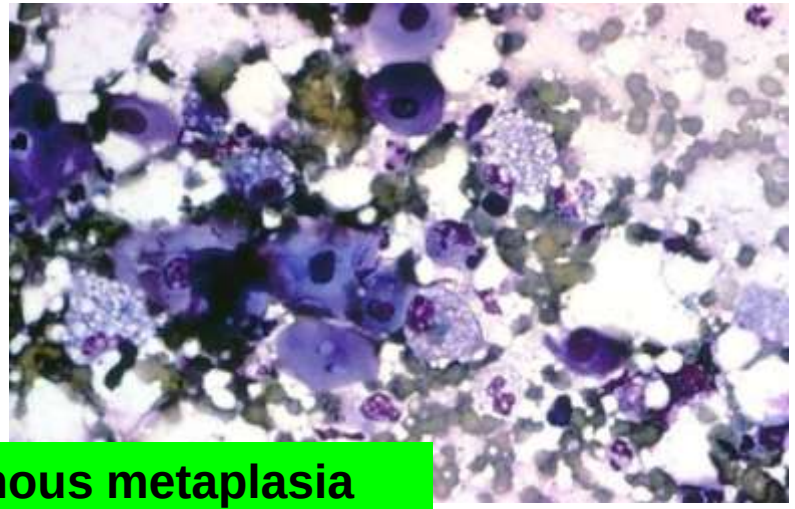
Canine Prostate – 76% cyto-histo correlation

- Same old issues for me :
  - Mixed processes (eg BPH and neoplasia)
  - Inflammation or necrosis masking neoplasia
  - Distinguishing TCC from adenocarcinoma (is it necessary?)

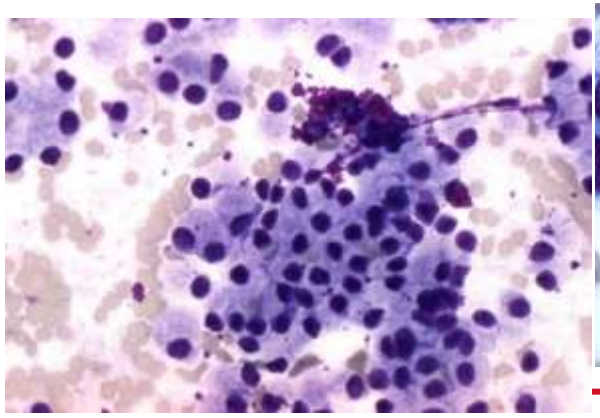
Prostatic disorders in the dog

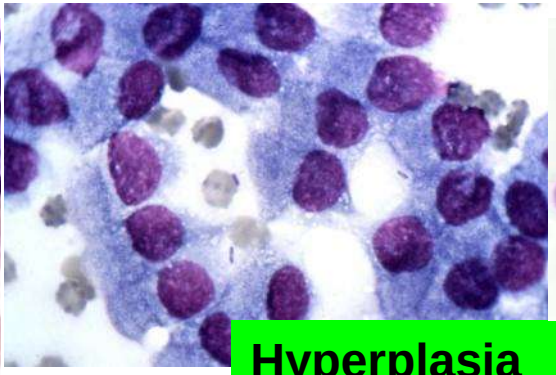
- Prostatic cysts
- Squamous metaplasia
- Prostatitis
- Hyperplasia
- Neoplasia
- A combination!

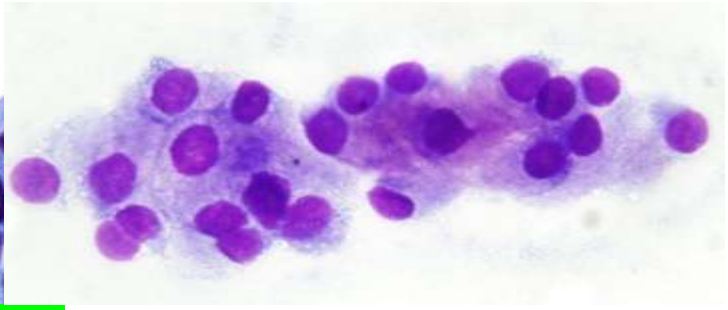




Squamous metaplasia

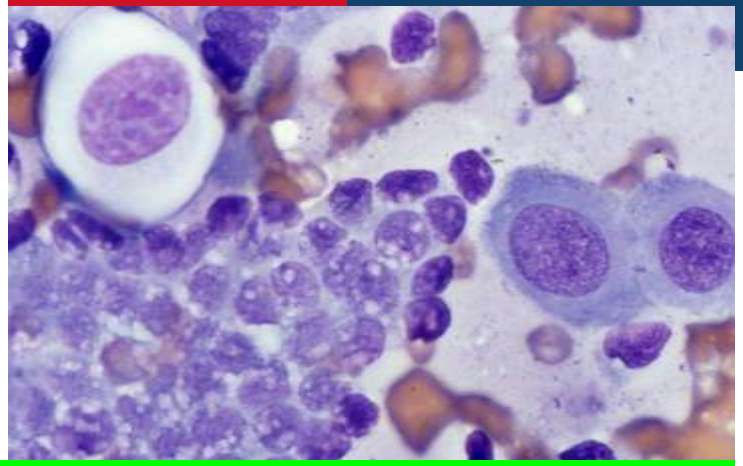




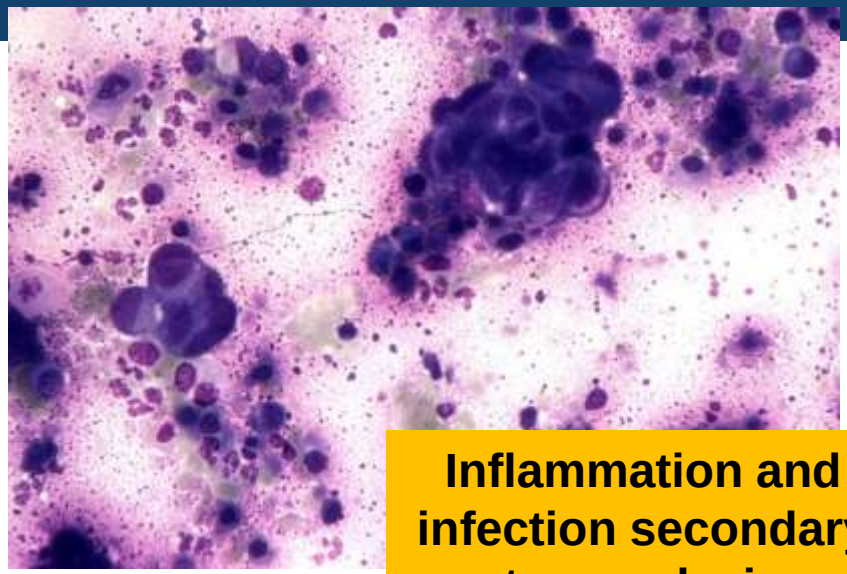


Hyperplasia

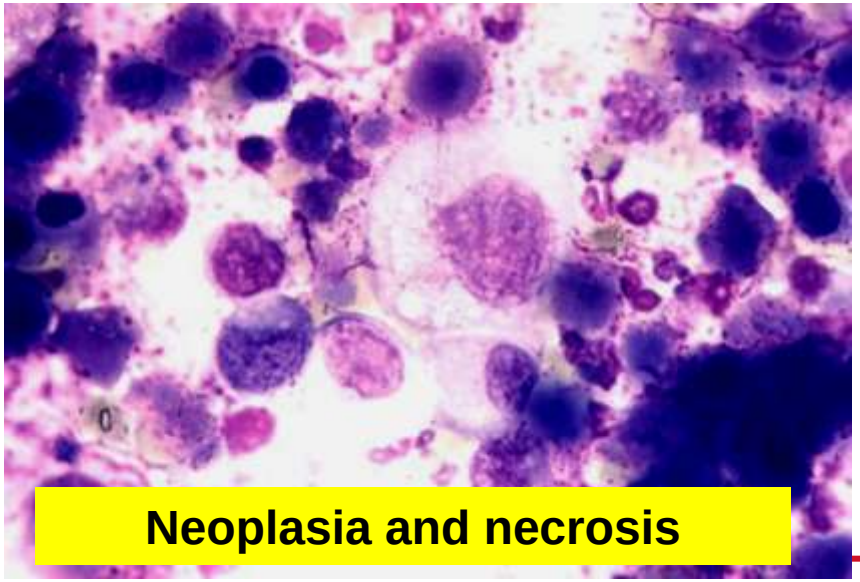
Complications in neoplasia



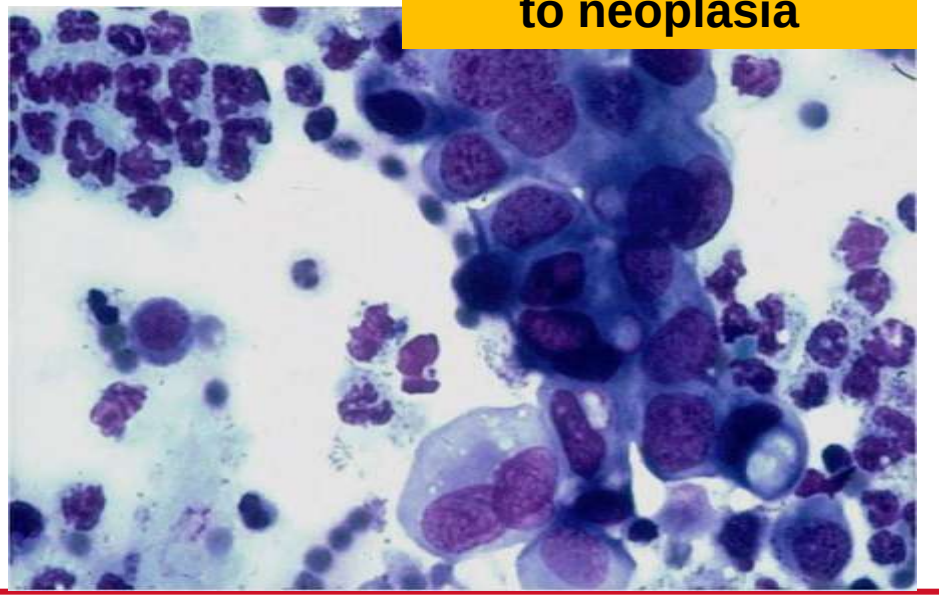
Neoplasia and hyperplasia



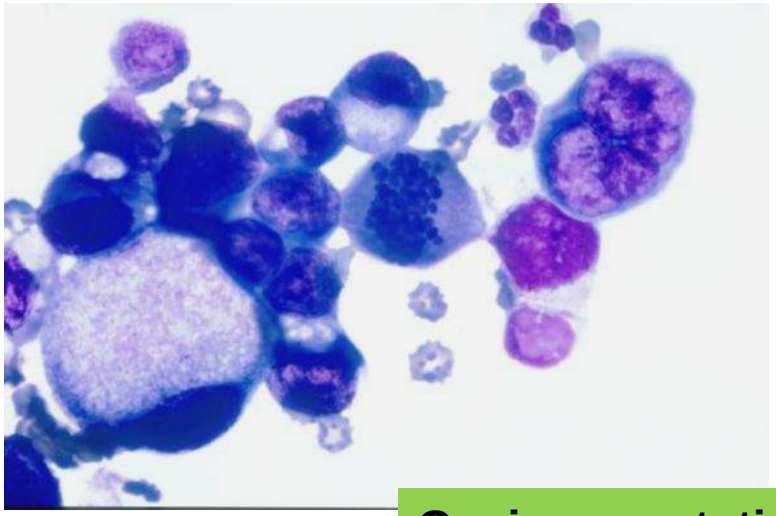
Inflammation and infection secondary to neoplasia

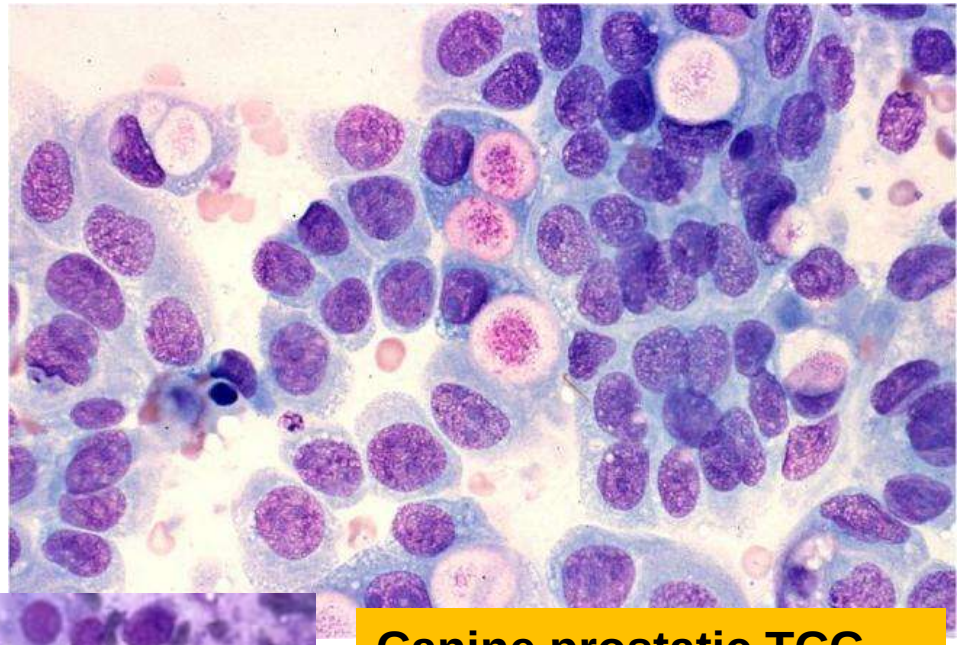


Neoplasia and necrosis

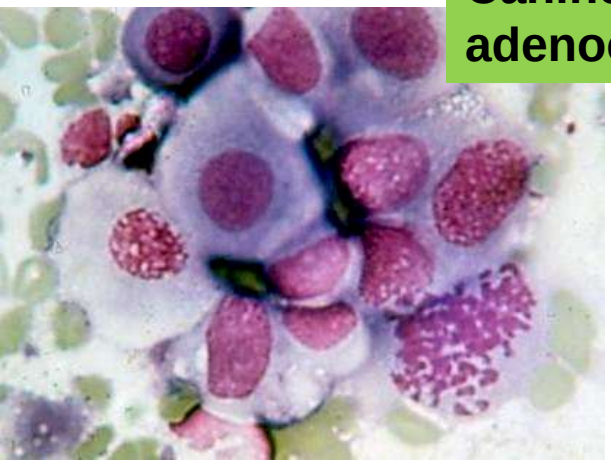


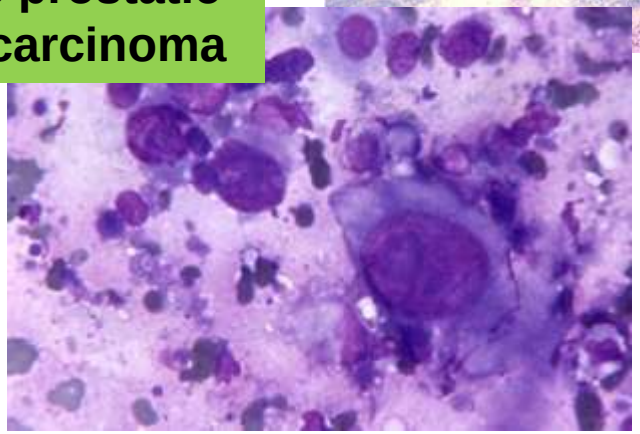
Prostatic neoplasia, apart from lymphosarcoma, is invariably TCC or adenocarcinoma. Do you have to know the difference?



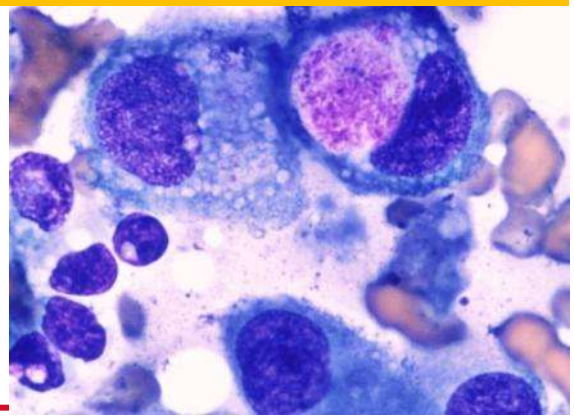


Canine prostatic adenocarcinoma





Canine prostatic TCC



More 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)?

Jeremy Allen DAFWA: ongoing investigation of haemoglobinuria in freshly calved dairy cows. Necropsy of a cow revealed enlarged pale liver, bloody fluid in peritoneum, thorax and pericardium. The urine was dark. Where are the trends?

TEST	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	REFERENCE VALUES
CK IU/L	345	176	344	158	**475**	**540**	<450
ALT IU/L	21	26	32	22	39	**50**	<40
GGT IU/L	9	6	4	17	13	**46**	<35
GD (GLDH) IU/L	39	4	5	12	**216**	**568**	<40
Total serum protein (biuret) g/L	71.7	**81.6**	68.5	72.8	**83.5**	**79.4**	67-75
Albumin g/L	32.4	34.4	38.0	36.8	**47.4**	**40.7**	27-39
Globulins g/L	**44**	**47.2**	30.5	36.0	36.1	38.7	24-40
A:G ratio	0.80	0.70	1.20	1.00	1.30	1.10	0.84:0.94
Haptoglobin mg/mL	**1.03**	**1.10**	0.51	0.36	**2.30**	**1.26**	<0.8
Total bilirubin µmol/L	**16**	**15**	**11**	6	8	**103**	<10
Total conjugated bilirubin µmol/L	3	3	2	1	3	**10**	<8.0
Cholesterol mmol/L	1.32	2.77	2.87	3.66	5.07	3.46	1.8-5.6
Urea mmol/L	8.8	4.3	4.8	4.5	5.8	9.3	1-10
Creatinine µmol/L	164	95	88	83	89	119	60-190
Calcium mmol/L	2.04	2.31	2.26	**2.83**	2.25	2.10	2.0-2.5
Inorganic phosphate mmol/L	1.54	**0.61**	**0.40**	**0.78**	**1.01**	**0.49**	1.2 – 2.3
Beta hydroxybutyrate mmol/L	**0.82**	**0.72**	0.37	0.33	0.35	0.32	<0.7
Iron µmol/L	**11.6**	12.3	**26.3**	**34.4**	**83.4**	**60.4**	12-23
Magnesium mmol/L	**0.56**	0.83	0.93	1.00	**1.28**	0.88	0.7-1.0

Jeremy Allen DAFWA: Post parturient haemoglobinuria in dairy cows

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	?	Clear
PCV L/L	**0.12**	0.24-0.46
Haemoglobin g/L	**47**	80-150
Erythrocytes $x10^{12}$/L	**1.74**	5-10
MCV fL	**70.7**	40-60
MCH pg	**27.0**	11.1-17.0
MCHC g/L	**382**	310-360
RDW (%)	**31.2**	12.0-27.0
Reticulocyte %	ND	0
Leukocytes $x10^9$/L	5.92	4-12
Neutrophils (seg.) $x10^9$/L	1.93	0.6-4.0
Neutrophils (band) $x10^9$/L	0	0-0.2
Lymphocytes $x10^9$/L	**2.33**	2.5-7.5
Monocytes $x10^9$/L	0.21	0.1-0.8
Eosinophils $x10^9$/L	1.02	0.2-2.4
Basophils $x10^9$/L	**0.43**	0-0.40
Platelets $x10^9$/L	103	100-800
Blood film:?		

Significant biochemical changes: increased liver enzymes and bilurubin (both unconjugated and conjugated), mainly increased iron, decreased inorganic phosphate, increased total protein, albumin and haptoglobin.

Combining history, biochemical and haematological values suggests post-parturient haemoglobinuria due to phosphorus deficiency. Necropsy of cow showed **acute hepatic necrosis, probably secondary to anaemia.** *Can you miss the low inorganic phosphate due to the haemolysis causing increases?*

Bovine Postparturient Hemoglobinuria: A Review of the Literature
P.S. Macwilliams, G.P. Searcy and J.E.C. Bellamy. Can. vet. J. 23: 309-312 (November 1982) - *Morphologically, the haemolytic anemia is characterized by evidence of intensified erythrogenesis. Polychromasia, anisocytosis, macrocytosis, basophilic stippling, sometimes Heinz bodies, reticulocytosis and increased numbers of nucleated erythroid cells are commonly seen in blood films*

A 13 years old male neutered poodle had a mild, dark, possibly bloody diarrhoea for 6 months. In the past 3 months the dog had lost about 15% of his BW and had developed a mild cough. The dog had a mild heart apical systolic murmur and a honking cough. It weighed 8.7 kg (body score 3 out of 5) and there appeared to be some sensitivity on abdominal palpation.

TEST	SAMPLE	REF VALUES
ALP u/l	100	<110
ALT u/l	**258**	<60
CK u/l	118	<200
Serum protein (biuret) g/l	**117.9**	54-73
Albumin (BCG) g/l	31.6	23-43
Globulins g/l	**86.3**	27-44
Bilirubin total µmol/l	2.4	1.2-8.1
Total cholesterol mmol/l	6.6	1.4-7.5
Glucose mmol/l	5.8	3.3-6.4
Urea mmol/l	**13.1**	3.6-10.0
Creatinine µmol/l	90	40-120
Calcium mmol/l	2.8	1.75-2.9
Inorganic phosphate mmol/l	**1.7**	0.8-1.6
Sodium mmol/l	**153**	137-150
Potassium mmol/l	3.7	3.3-4.8
Chloride mmol/l	110	105-120
Bicarbonate mmol/l	19	17-21

TEST	SAMPLE	REF VALUES
Plasma appearance	Clear	Clear
PCV l/l	0.41	.37-.50
Plasma protein g/l (refractometer)	**117**	55-75
Haemoglobin g/l	**157**	100-150
Erythrocytes $x10^{12}$/l	6.35	5-7
MCV fl	65	60-75
MCHC g/l	**383**	300-360
MCH pg	24.7	20-25
Leukocytes $x10^9$/l	**6.1*** (corrected)	7-12
Neutrophils (seg.) $x10^9$/l	4.95	4.6-9.36
Neutrophils (band) $x10^9$/l	0.0	0-0.42
Lymphocytes $x10^9$/l	**0.73**	0.91-3.6
Monocytes $x10^9$/l	0.24	0.21-0.96
Eosinophils $x10^9$/l	0.18	0.14-1.2
Basophils $x10^9$/l	0.00	0-0.36
Platelets $x10^9$/l	300	300-700
Reticulocyte % (uncorrected)	**1.6**	0-1.5

Blood film: ***Erythrocytes*** - slight polychromasia; 6 nucleated erythroid cells per 100 leukocytes ***Leukocytes*** – neutrophils appear to have prominent granulation; there are scattered hyperbasophilic lymphoid cells.
*Leukocytes corrected for the level of circulating nucleated erythroid cells

Colour and appearance of **Urine** (voided) : light amber and slightly cloudy	Glucose: -ve
Specific gravity: 1.020	Ketones: -ve
Protein (SSA): **3+**	Blood: **2+**
pH: 7.0	Bilirubin: -ve
Microscopic findings (centrifuged): some lipid, 10 erythrocytes per high power field, 3 leukocytes per HPF, scattered transitional and squamous epithelial cells, and occasional struvite crystal.	

Likely conclusions: apart from the clinical indications for cardiorespiratory and gut disease, the dog has indications of multisystem disease (liver, kidney). There are also haematopoietic disturbances that could be further investigated. Finally, the hyperglobulinaemia is serious enough to investigate further to see whether it is related to ongoing inflammation or perhaps neoplasia (affecting the lymphoid system)

(**postscript:** the dog was diagnosed with **Multiple Myeloma** on the basis of the hyperglobulinaemia being composed of a narrow based gamma globulin (clonal in nature) and bone marrow identification of neoplastic plasma cells. In hindsight the hyperbasophilic lymphoid cells were plasmacytic in nature, which was confirmed by examination of similar cells in a buffy coat preparation of peripheral blood (ie there was plasma cell leukaemia). The imaging of the abdomen showed splenomegaly and mild hepatomegaly. A FNA of the spleen revealed numerous neoplastic plasma cells (bone marrow was then taken after this result). The cardio-respiratory complaints were not investigated further. There was definitely some blood in the faeces, but this in addition to some bleeding in the urine was thought to be related to the neoplastic condition. The proteinuria could have been related to the neoplastic condition (Bence Jones protein) and/or some level of renal disease in an aging dog)

Possible reasons for changes : the most significant biochemical change is the hyperglobulinaemia. Whilst some of this might be due to dehydration (there is also a mild hypernatraemia and the dog has had protracted diarrhea)), there is likely to be another reason why the globulins are elevated. The mild elevation in ALT indicates some active hepatocellular damage. The haematological results are confusing. There is no anaemia (in fact there is an elevated Hb), but the reticulocyte count is marginally elevated suggesting some mild regeneration. Perhaps this dog has a compensating anaemia, especially if there has been continued bleeding into the gut. However, one would expect to see loss of iron over a prolonged period perhaps leading to microcytosis and hypochromasia (in fact there is an elevated MCHC – perhaps there has been some haemolysis?). Additionally, the level of circulating nucleated erythroid cells is inappropriate for such a mild reticulocytosis. Perhaps this might indicate bone marrow or splenic disease (or could respiratory disease be contributing)? The marginal leukopaenia, lymphocytopaenia and lowish neutrophils (still within the reference interval) are also difficult to fathom. The lymphocytopenia might be partly related to stress, but there are no other clinical findings to support that. Also, commonly in regenerative anaemia you get a mild neutrophilia. Perhaps there is a bone marrow disturbance? The neutrophils with prominent granulation might indicate mild toxic change whilst the hyperbasophilic lymphoid cells could be reactive or even neoplastic.
The urine changes suggest mild bleeding that could be contributing to the compensated anaemia if it has occurred over a prolonged period (although, once again, this is external blood loss and could lead to non-regenerative anaemia). There could also be some free haemoglobin in the urine as the blood strip reading doesn't seem to match completely with the sediment of 10 erythrocytes per HPF. The proteinuria is also significant at the specific gravity reading. The true SG reading is probably in the teens (falsly elevated by the proteinuria) and may indicate some interference with concentrating ability, especially if the animal is dehydrated. The mild azotaemia could be pre-renal in origin (eg dehydration, bleeding in the gut) but one cannot exclude the possibility of a renal component (usually renal azotaemia does not develop until there is close to complete loss of concentrating ability).
Likely conclusions: apart from the clinical indications for cardiorespiratory and gut disease, the dog has indications of multisystem disease (liver, kidney). There are also haematopoietic disturbances that could be further investigated. Finally, the hyperglobulinaemia is serious enough to investigate further to see whether it is related to ongoing inflammation or perhaps neoplasia (affecting the lymphoid system)
(**postscript:** the dog was diagnosed with Multiple Myeloma on the basis of the hyperglobulinaemia being composed of a narrow based gamma globulin (clonal in nature) and bone marrow identification of neoplastic plasma cells. In hindsight the hyperbasophilic lymphoid cells were plasmacytic in nature, which was confirmed by examination of similar cells in a buffy coat preparation of peripheral blood (ie there was plasma cell leukaemia). The imaging of the abdomen showed splenomegaly and mild hepatomegaly. A FNA of the spleen revealed numerous neoplastic plasma cells (bone marrow was then taken after this result). The dog is now on specific therapy for the condition. The cardio-respiratory complaints were not investigated further. There was definitely some blood in the faeces, but this in addition to some bleeding in the urine was thought to be related to the neoplastic condition. The proteinuria could have been related to the neoplastic condition (Bence Jones protein) and/or some level of renal disease in an aging dog)

An 11 years-old female thoroughbred is presented with inappetence, extreme weight loss, soft stools and oedema of the head and neck. The period of illness was in excess of six weeks.

TEST	SAMPLE	REF VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.37	0.32-.52
Plasma protein (refract) g/L	37	58-84
Fibrinogen g/L	3	2-4
Leukocytes $x10^9$/L	8.6	6.0-13.0
Neutrophils (seg.) $x10^9$/L	6.1	2.5-6.9
Neutrophils (band) $x10^9$/L	**0.3**	0-0.24
Lymphocytes $x10^9$/L	**1.5**	1.6-3.4
Monocytes $x10^9$/L	0.5	0-0.8
Eosinophils $x10^9$/L	0.2	0-0.96
Basophils $x10^9$/L	0	0-0.36
Blood film: normal		

Urinalysis (catheterised)	PH: **5.0**
Appearance: cloudy	Glucose: -ve
Colour: yellow	Ketones: -ve
Specific gravity: 1.030	Blood: trace
Protein: -ve	Bilirubin: -ve
Microscopic findings: much mucus, **5-8 erythrocytes** and 1 leukocyte per high powered field (HPF – 40x objective). Some fat droplets	

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	292	<400
AST IU/L	221	<400
GGT IU/L	10	<30
GD (GLDH) IU/L	1.9	0.9-4.7
Total serum protein (biuret) g/L	**29**	60-76
Albumin g/L	**8**	29-38
Globulins g/L	**20**	26-40
A:G ratio	**0.4**	0.62-1.46
Total bilirubin µmol/L	**70**	<50
Glucose mmol/L	4.8	4.5-6.3
Urea mmol/L	**25.1**	3.7-8.2
Creatinine µmol/L	**180**	87-149
Calcium mmol/L	**2.0**	2.8-3.3
Inorganic phosphate mmol/L	1.5	0.8 – 1.8
Sodium mmol/L	**126**	132-150
Potassium mmol/L	4.8	2.8-5.0
Chloride mmol/L	101	99-110
Bicarbonate (TCO_2) mmol/L	**14**	24-32
Anion gap	15.8	12-20

Oral glucose tolerance Test (Patient and Control horse)
Baseline: 4..8 (Patient - P) 5.1 (control horse - C)
Every 30 minutes up to 5 hours:
P: **4.8 5.2 5.3 5.3 5.2 4.8 4.7 5.0 4.9 4.8**
C: 6.0 8.0 8.1 8.0 7.9 7.8 6.2 4.8 5.1 4.7

Likely conclusions: there is a strong suggestion that there is small intestinal disease causing significant protein loss (and consequent calcium) (protein losing enteropathy) and malabsorption. Lymph may be being lost at the same time. The oedema seems to be related to the marked hypoalbuminaemia.
(**Postscript:** This animal had biopsies taken which revealed **lymphocytic inflammatory bowel disease.** No infectious cause was detected. The animal was destroyed as it did not respond to antibiotics or anti-inflammatories)

Inflammatory Bowel Disease in Horses. Karen A. Kalck. Vet Clin Equine 25 (2009) 303–315. *Lymphocytic/plasmacytic enteritis is characterized by excessive infiltration of lymphocytes and plasma cells in the lamina propria of the gastrointestinal tract with the absence of granulomatous change. There is no age, breed, or sex predilection for this form of IBD. It has been suggested that this condition may be an early stage of intestinal lymphosarcoma.*

Possible reasons for changes: the marked panhypoproteinaemia (both albumin and globulins) is more likely to be due to loss (gut, urine) rather than to inappetence alone. Chronic liver failure can also contribute to hypoproteinaemia (however, hypoglobulinaemia is not always present). The mild lymphopenia could be due to corticosteroid release ('stress'), but since there are no other signs of stress perhaps it is due to lymph loss (via gut?). The mild hyperbilirubinaemia could be due to inappetence (in that case all of the increase will be unconjugated). The mild to moderate azotaemia (affecting urea more than creatinine) could be prerenal as the urine SG and lack of proteinuria does not suggest a renal component. The borderline haematuria is probably due to the catheterisation. The hypocalcaemia is probably due to the low albumin (since total calcium has been measured which includes protein bound as well as ionised forms). Correction for the low albumin (measured total calcium + [average albumin {30 for the horse} – alb value divided by 40] = 2.0 + 22/40 = **2.55**), however, still gives a low calcium (2.55) which may mean that there is a true loss along with the protein (in the gut?). The mild hyponatraemia could be related to dilution because of the expanded ECF (oedema). The MAc without increased anionic gap could indicate secretory mechanisms (ie loss of bicarbonate in diarrhoea). This may account for the acid urine (compensatory mechanisms. The oral glucose tolerance test (shows a flat curve compared to the control horse) could suggest a problem with absorption from the small intestine.

Likely conclusions and further investigation: there is a strong suggestion that there is small intestinal disease causing significant protein loss (and consequent calcium) (protein losing enteropathy) and malabsorption. Lymph may be being lost at the same time. The oedema seems to be related to the marked hypoalbuminaemia.

Further investigation is really aimed at confirming the pathological process and its likely cause. Image analysis might be done to identify infiltrative disease of the small intestine (rectal palpation could be tried also). You may decide on endoscopy and the taking of biopsy material from different areas of the small intestine for histopathological examination. Intestinal washings could be used for culture if required. Peritoneal fluid analysis may be done because it is easy and may detect a disease process that has spilled over from the small intestine. You may wish to repeat some the abnormal laboratory results to ensure that they are persistent

(**Postscript:** This animal had biopsies taken which revealed lymphocytic inflammatory bowel disease. No cause was detected. The animal was destroyed as it did not respond to antibiotics or anti-inflammatories)

A 2 years old male Springer spaniel with diarrhoea, inappetence and weight loss of 6 weeks duration.

BIOCHEMISTRY	SAMPLE	REFERENCE INTERVAL
Serum protein (refract.) g/L	**17**	50-70
Albumin (EPG) g/L	**8.8**	23-39
α globulins (EPG) g/L	**2.9**	7-16
β globulins (EPG) g/L	**4.7**	9-16
γ globulins (EPG) g/L	**0.6**	4-12
Calcium mmol/L (uncorrected)	**1.6**	2.1-2.9

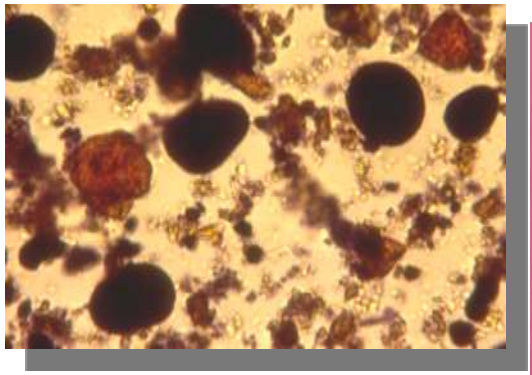
***Other tests*:**
Serum trypsin/trypsinogen like Immunoreactivity (TLI) µg/L: 12 (reference interval 5-35)

FAECAL ANALYSIS:
Trypsin (really all faecal proteases): **5 Azoalbumin units** (reference interval >7)
Blood: present
Smears for undigested/ unabsorbed food particles: no increase in starch, undigested muscle or undigested fat (unsplit, primarily triglycerides); **large increase in unabsorbed fat (split, primarily free fatty acids)**

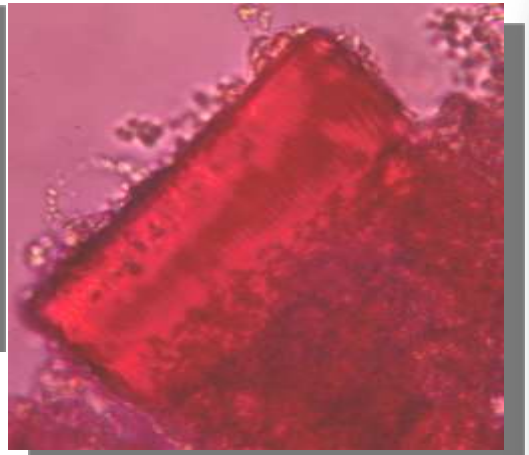
HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	clear	Clear
PCV L/L	0.49	0.37-0.50
Plasma protein g/L	**23**	55-75
Haemoglobin g/L	**162**	100-150
Erythrocytes $x10^{12}$/L	**7.4**	5-7
MCV fL	66	60-75
MCHC g/L	331	300-360
Leukocytes $x10^9$/L	**14.8**	7-12
Neutrophils (seg.) $x10^9$/L	**11.2**	4.1-9.4
Neutrophils (band) $x10^9$/L	0	0-0.24
Lymphocytes $x10^9$/L	3.1	0.9-3.6
Monocytes $x10^9$/L	**0**	0.2-1.0
Eosinophils $x10^9$/L	0.5	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film: normal		

A blast from the past? Who does this type of faecal analysis today?

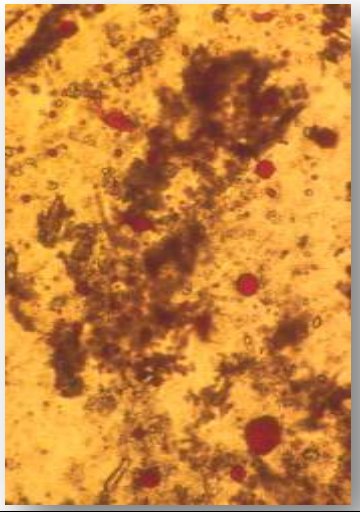
Preparing faeces for staining for undigested and unabsorbed food particles in the dog

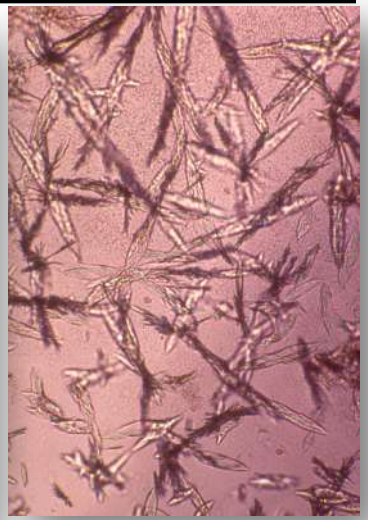


Amylorrhoea



Creatorrhoea





Steatorrhoea – undigested and unabsorbed

Likely main conclusion: the persistent diarrhoea and evidence of malabsorption from faecal tests and TLI suggest that the hypoproteinemia is due to gut disease.
Further investigation could involve diagnostic imaging, intestinal absorption tests, endoscopic examination and gut biopsies The hypoproteinemia could be investigated by injecting protein bound to radioactive chromium, and checking for losses in the feces.
Diagnosis and postscript: *Malabsorption and protein losing enteropathy due to severe inflammatory bowel disease (IBD).* IBD is a poorly understood, probably multifactorial condition of dogs and cats and, depending on intensity, can cause a variety of clinical signs relating to dysfunction of the small intestine.

Possible reasons for changes and likely conclusions: the dog has a significant hypoproteinaemia (all classes), which in this case is most likely due to an enteropathy (faecal tests and the clinical signs suggest this) (**conclusion)**. End stage liver disease can cause a hypoproteinaemia affecting most protein groups, although gamma globulins are not usually affected and may actually be increased. Severe starvation may cause a hypoproteinaemia. Kidney disease may cause hypoproteinaemia but this commonly involves only albumin (globulins are generally too large to be lost by the glomeruli or tubules). In fact some globulins may increase in the blood. The borderline hypocalcaemia (once corrected) in this case is possibly due to loss of protein bound calcium through damaged gut (most kits detect total calcium which includes protein bound and free ionised forms). The corrected value is 2.2 mmol/L. Increased unabsorbed fat in the faeces suggests an enteropathy as most cases of malabsorption are due to enteric disease (**conclusion)**. The slightly decreased trypsin activity has no significance in this case as the smears for food particles do not suggest maldigestion. This is also supported by the normal TLI (less than 2.5 μg/L is diagnostic for maldigestion due to EPI). The TLI has replaced the faecal trypsin test for maldigestion due to EPI. The positive faecal blood may indicate mild leakage associated with a gut disease. However, the test is not terribly reliable as it can give false positives due to the presence of other substances having peroxidase activity. The mild neutrophilia in the absence of other white cell changes may suggest limited inflammatory demand, perhaps related to gut disease. The monocytopaenia has no significance in any circumstances. The elevated Hb and erythrocyte count can be best explained by haemoconcentration. Commonly, non-regenerative anemia occurs in chronic bowel diseases but perhaps this one has not been occurring for long enough.

Likely main conclusion: the persistent diarrhoea and evidence of malabsorption from faecal tests and TLI suggest that the hypoproteinemia is due to gut disease.

Further investigation could involve diagnostic imaging, intestinal absorption tests, endoscopic examination and gut biopsies The hypoproteinemia could be investigated by injecting protein bound to radioactive chromium, and checking for losses in the feces.

Diagnosis and postscript: Malabsorption and protein losing enteropathy due to severe inflammatory bowel disease (IBD). IBD is a poorly understood, probably multifactorial condition of dogs and cats and, depending on intensity, can cause a variety of clinical signs relating to dysfunction of the small intestine.

One year old female neutered German shepherd.
PRESENTING COMPLAINTS: Weight loss, normal to increased appetite, soft voluminous feces. Gradually developing over 3 months.
LABORATORY RESULTS:
BIOCHEMISTRY:
Trypsin/trypsinogen like
Immunoreactivity (TLI) μg/L: 1.5 (reference interval 5-35)
FAECAL ANALYSIS:
Trypsin (really all faecal proteases): 2.6 Azoalbumin units (reference interval >7)
Blood: absent
Smears for undigested/ unabsorbed food particles: no increase in starch or unabsorbed fat (split, primarily free fatty acids), Large increases in undigested muscle and undigested fat (unsplit, primarily triglycerides).

Exocrine pancreatic insufficiency for comparison with previous case of malabsorption - How many of you are still seeing EPI?