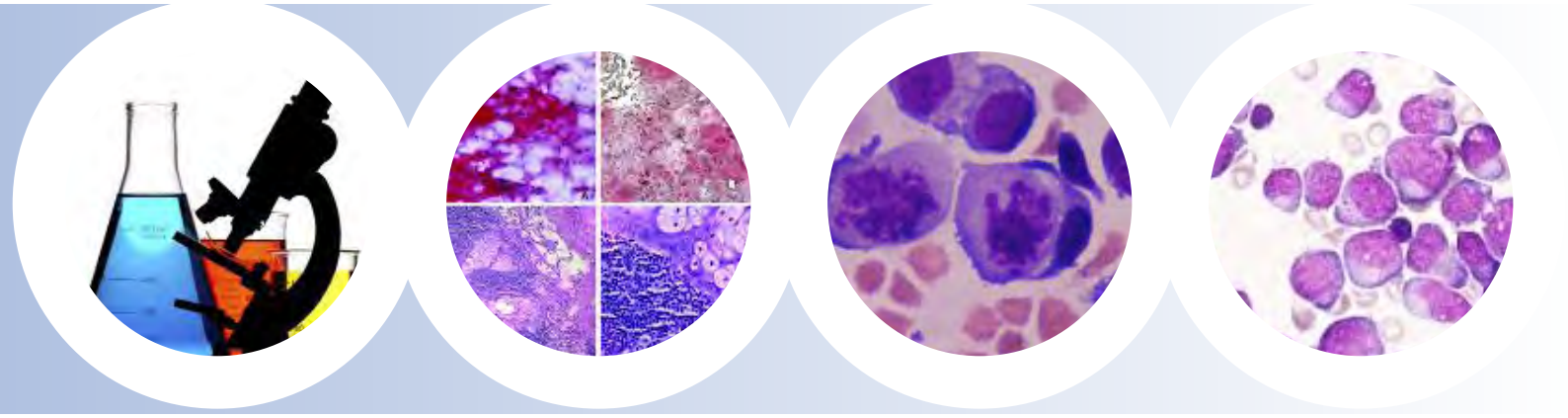
World Small Animal Veterinary Association

Cytology in practice

Course notes



Lecturer

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DVM, MRCVS, FHEA, Diplomate ACVP

Senior Lecturer in Clinical Pathology

Royal Veterinary College, London

March 9th , 2014 (Saturday)

CROWNE PLAZA HOTELS & RESORTS

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Curriculum vitae

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Balázs is a graduate of Szent István University, Faculty of Veterinary Science, Budapest, Hungary. After graduation he completed an internship in small animal internal medicine and stayed on as a clinical instructor in the Department of Internal Medicine at his alma mater. He won a Fulbright scholarship to the University of Missouri-Columbia, College of Veterinary Medicine, Department of Medicine & Surgery. After, he completed a residency program in veterinary clinical pathology at Kansas State University. He joined the Royal Veterinary College in January 2006 and is a Diplomate of the American College of Veterinary Pathology (Clin Path). His main priorities are striving for excellence in teaching and providing the diagnostic service in hematology, biochemistry and cytology. He has also developed and runs a highly successful residency training program for board certification in veterinary clinical pathology (given by the ACVP) at the RVC to train the next generation of clinical pathologist.

CYTOLOGY IN PRACTICE

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Introduction

The primary goal of a cytologic examination is to gain valuable information about a lesion, thus it has to be considered as a SCREENING tool: while in many cases it can give definitive diagnoses, in others it will help narrow our differentials, or justify further, potentially more invasive/expensive procedures.

Indications

Cells may be collected by a number of methods including aspiration, imprinting, washing, squashing and scraping. Fine needle aspiration is most commonly performed on skin masses and enlarged lymph nodes. Fluid analysis can be performed on joint fluids, body cavity fluids/effusions, respiratory tract washings (TTW, BAL), or cerebrospinal fluids. With the advance of ultrasound guidance, organs (e.g. spleen, liver, kidney, mesenteric lymph nodes) and lesions deep within the body (e.g. mediastinal mass) can also be sampled. Naturally, different sampling techniques and different sources of samples may have different limitations or difficulties in getting nice samples.

Cytology vs. histopathology

Cytologic sampling/examination is relatively simple, quick, inexpensive, minimally invasive, and has quicker turn around time then biopsies when sent off to a pathologist. However, with good basic training and a quality microscope, many straight forward lesions can be diagnosed in house, right next to the patient. Furthermore, when cell detail is critical (e.g. round cell tumours), it can even out perform routine histopathologic examination. Biopsy procedures are more time consuming, more expensive

(sterile procedure, anaesthesia) with a slower turn around time; however it will provide valuable information about tissue architecture, which can be essential for diagnosis. Also grading, invasion etc. can only be evaluated via the latter procedure. Unusual lesions (e.g. poorly differentiated neoplasia) commonly need immunohistochemical examination, which is currently much more readily available for tissues than cells on a smear. Lastly, the representativeness of the sample is important. While entire masses excised (excisional biopsy) will obviously be the most representative samples, in my experience, properly acquired aspirates can be actually more representative then tru-cut biopsies.

Common mistakes during sampling

For a long time, FNAs were performed via aspiration, as the name implies. However, creating vacuum with our syringe within the tissue will hurt our samples more time then help! First always attempt to only use a small gauge needle (22-24G) without an attached syringe, and gently advance it (with one hand) into the well stabilized (with the other hand) lesion, then pull out partially (still within the mass), redirect, and advance more deeply into the mass again, multiple times to increase representativeness of the sample. While it is somewhat counterintuitive, but very small amount of material is needed to prepare nice smears – literally just the content within the length of the needle is enough. We have to avoid “contaminating” our sample with blood, as it really makes the evaluation much more difficult. Blood will represent the “haystack”, while the cells of interest will be the “needles”. Furthermore, blood doesn’t only have erythrocytes, but neutrophils will come with it, again making judgement more difficult.

Actual aspiration should only be attempted, if couple of attempts with the needle only failed (e.g. poorly exfoliating neoplasia). As mentioned above, very small amount of material is needed, thus if fluid has already appeared in the hub of the needle, it is likely too much (unless it is an abscess or cyst type lesion)!

Common problem is caused by large necrotic areas in the middle of lesions (e.g. malignant neoplasia). Just simply going for the middle of the lesion (without redirecting the needle) will provide highly diagnostic slides of necrosis, but not necessarily for the underlying pathology. Therefore, if we suspect a mass with a “soft middle”, make sure to redirect the needle into the more outer “walls” of the lesion as well.

Acquiring highly diagnostic material from the lesion however is only the first challenge. Then comes the preparation of the smears, which is another common source of problems. Many people will try to spread the cells throughout the entire glass slide via forceful spraying action, which unfortunately only creates droplets of fluid on the slide, which will be very thick after drying, and nothing in between. The primary goal during slide preparation is the spreading of the material to create thin areas where cells will flatten out (cell should appear as fried eggs vs. a boiled egg cut in half) without much lysis of them. The best practice is the attach the syringe (e.g. 5 mL) to our needle containing the valuable sample, and very gently squirting it out to one end of the slide. Then another slide is placed at 90°, creating a square between the two slides. After the material has started spreading within the "square", the top slide needs to be glided along the bottom slide. The amount of pressure applied is something that needs fine tuning, which can be greatly achieved by examining our own prepared slide. Too little pressure will create too thick preparations, while too much pressure will lysed many cells (especially the fragile ones, such as neoplastic cells). Only intact cells can be reliably assessed, thus our goal is to have many intact cells, with adequate spreading as mentioned above.

Imprints can also provide excellent preparations, if the following tips are followed. Try to create a fresh cut surface (e.g. cutting the excised tissue in half – will also aid the penetration of formalin), which HAS to be blotted dry with a paper towel. Without this critical step, we are only "imprinting" the tissue juices and blood on the surface, but not the cells. On the other hand, with the dried, granular appearing surface of the mass will exfoliate really well even with gentle pressure, which is important to avoid to thick specimens (with too much pressure). Multiple imprints can be done on one glass slide, dependent on the size of the cut surface of course.

Even though it sounds very convenient to imprint an excised tissue right above the open formalin jar (so we can just drop it in when we are done), unfortunately even just the fumes of formalin will completely destroy cytologic preparations! Never have open formalin jars anywhere close to blood smears or cytologic preparations! Even closed jars, packed together with smears for mailing will have detrimental effects, thus at minimum double pack each, separately.

As most lab samples commonly kept in the refrigerator until processing or shipping, it is not uncommon for the glass slides (blood smears, cytologies) to end up in the fridge as well. However, those with glasses will know well what happens to cold glass in a warm room! Water condensation on

the glass will completely destroy the cells! Just air drying smears (but also avoid hot air of a hairdryer) will preserve the cells very well, so there is no need for fixation or refrigeration.

Lastly, but not the least, good communication with the pathologist (both ways) is essential to increase the efficiency of our cytologic diagnostics. This process starts with properly labelled and packaged slides, and providing adequate information about the case. It is a common myth to try to keep the pathologist "blinded" to avoid bias. Most well trained/certified clinical pathologists will only report what is present on the slides, however in our comment, we can give much better information if we know more about the lesion. In other works, letting me know of the dog's hypercalcemia will not make me call a lymph node aspirate a lymphoma, unless I am certain based on the cytologic picture. However, if I can't make such a conclusion on the submitted slide, I may still comment, that while this slide was not diagnostic of lymphoma, but further tests (e.g. excision of the node) could be considered in face of such a biochemical finding to make sure that an important diagnosis is not missed! If e.g. the differential diagnoses are provided, it is easy to comment, whether the cytologic picture is consistent or inconsistent with each of the listed differentials.

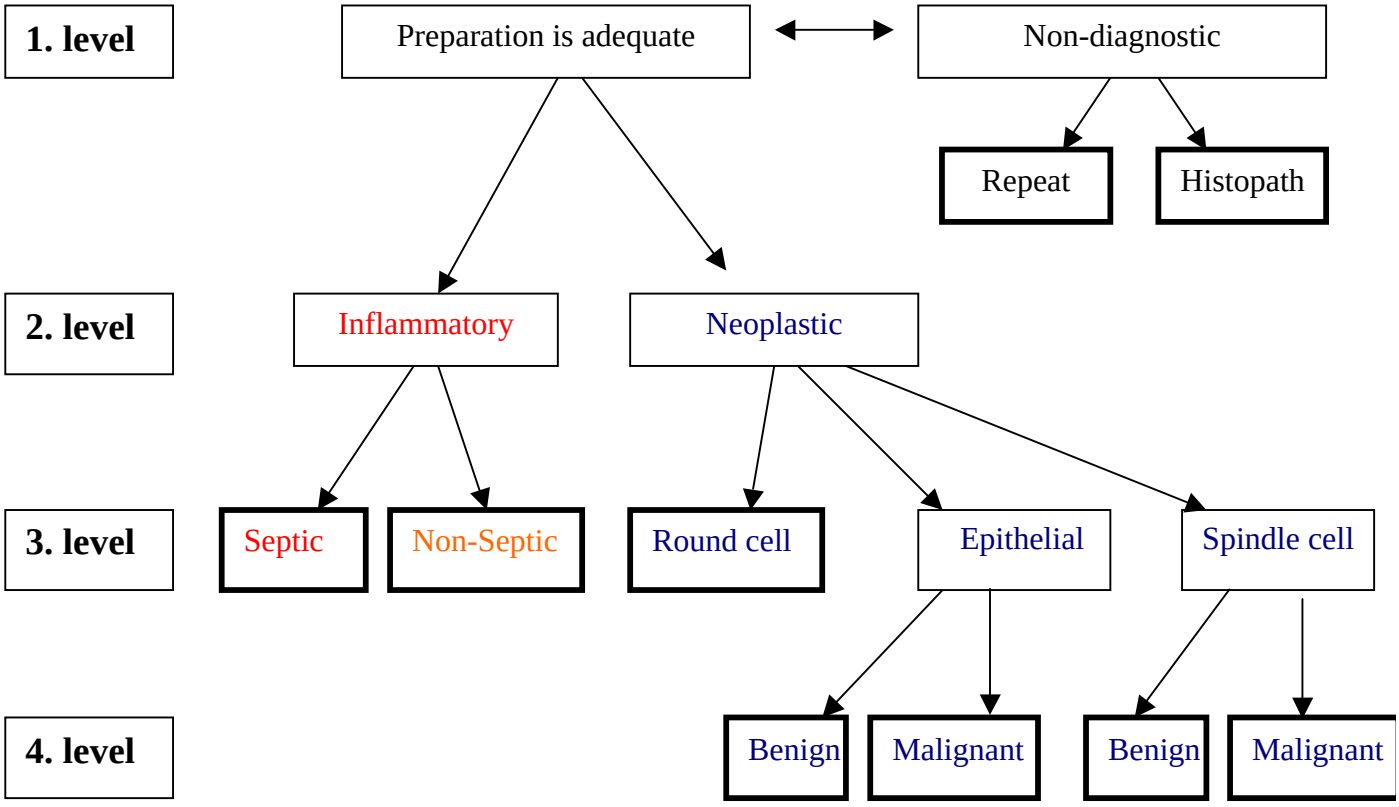
Even such "simple" information, whether the lesion is from the surface of the skin, or being dermal (moves with the skin) or subcutaneous (skin moves above it), makes a significant difference in the interpretation. Another simple information commonly hidden such as signalment can make a big difference. With a large mass in the abdomen, neoplasia of a retained testicle can have similar cytologic picture as an ovarian mass, thus just knowing the sex of the animal will help greatly in the evaluation of the sample.

If one is looking for metastasis, knowing the primary tumor type will greatly enhance the search for the metastatic cells.

Lastly, a common problem originates from the fact, that when a lesion is right in front of our eyes, it is easy to describe it too superficially, but we have to keep in mind that the pathologist will not have the same luxury. Favourite example: abdominal mass – yes, it is very obvious when somebody is standing in front of a radiograph with a giant mass in the abdomen, but by the time it comes to us, the question arises: are we talking about an intraabdominal mass, or a skin mass on the abdomen ☺.

SYSTEMATIC APPROACH TO CYTOLOGY SPECIMENS

1. Evaluate the quality of the preparation
    a. Adequate amount of cells
    b. Adequate spread (thin areas)
    c. Adequate staining
2. Scan the entire slide on low magnification (4-10X) to find the best areas to spend you time on!
3. Assess the cellular arrangement on low power – clusters of cells, sheets, individual cells, etc…
4. Try to decide if it is inflammatory or neoplastic (or normal, if the tissue sample is such, that this is an option)
    a. Inflammatory
        i. Identify the cell types involved (neutrophils, macrophages, eosinophils, lymphocytes, etc…)
        ii. Decide if **septic** or **not**
    b. Neoplastic – try to decide which of the 3 main categories
        i. Round cell tumor – histiocytoma, MCT, lymphoma, plasma cell tumor …
            - Biologic behavior is more specific to the type then cytologic features
        ii. Epithelial tumor – look for features of malignancy, key word: pleomorphism!
            1. **Benign** (e.g. adenoma)
            2. **Malignant** (e.g. carcinoma)
        iii. Mesenchymal/spindle cell tumor – features of malignancy…
            1. **Benign** (e.g. fibroma)
            2. **Malignant** (e.g. sarcoma)



Limitations and things to keep in mind during interpretation

- A perfect cytologic diagnosis may not represent the actual lesion (e.g. squamous cell carcinoma commonly has necrotic, inflammatory and possibly septic areas, especially within the oral cavity).
- Spindle cell tumours are a cytologic minefield. Fibroblasts within granulation tissue may appear more atypical then cells of a well-differentiated fibrosarcoma. Interpretation of many lesions with spindle cells needs considerable experience. This is the type of lesion where most commonly histopathologic examination is required (and suggested in the cytology conclusion) for a reliable diagnosis. Nonetheless, these cause the most frustrations in clinicians: “why didn’t I just biopsy it?” It is important to keep in mind, that cytology is a screening tool! Before aspirating the lesion, we didn’t know anything about it (could have been an abscess, MCT, lipoma, etc), thus getting this far is actually very helpful within the context of our diagnostic investigation, even if the final diagnosis is not reached via cytology.
- Concurrent presence of tissue and inflammatory cells: inflammatory lesions can easily contain epithelial (e.g. nasal epithelium) or spindle cells (fibroblasts), while malignant tumours (both carcinomas and sarcomas) can have necrotic and inflammatory areas within the mass. The dilemma is further intensified by the fact, that epithelial cells will commonly become dysplastic, when the tissue is inflamed, giving cytologic features of malignancy to the cells. There is a grey zone between dysplasia and neoplasia when cells are examined via light microscopy. Squamous, respiratory and transitional epithelia, and mesothelium are common to appear “malignant” for the untrained eye due to the dysplastic/reactive features. Spindle cells are similarly challenging; fibroblast walling of an abscess will also look quite “atypical”, and easily misclassified as malignant without experience. Thus, whenever tissue cells and inflammation are both present, it is wise to seek a second opinion, and in fact these cases may also be reported out by the pathologists with the recommendations for histopathologic examination.
- Mammary masses – only aspirate to confirm whether it is a mammary mass, and not a mast cell tumour, abscess, etc… Unfortunately mammary tumours can have several different areas (normal, dysplastic, benign, malignant) within the same mass, and cytologic pleomorphism does not correspond well with the true biologic behaviour. Only stromal invasion and evidence of metastasis appears to correlate with survival, both of which needs to be evaluated via histopathologic examination.

- Cytologic evaluation for malignancy relies on cellular pleomorphism – the more atypical and variable the cells are, the more likely to be malignant. However, certain highly malignant tumours may appear very uniform (e.g. thyroid carcinomas), thus location, and in depth knowledge of certain tumours can be vital in order not to miss malignancy.
- Certain malignant cell types (e.g. transitional cell tumours) easily metastasize, thus aspiration may seed the neoplastic cells into surrounding tissues (e.g. aspirating prostate through the abdominal wall).
- Bacteria – stain precipitation can mimic bacteria, especially cocci. With well kept stain, bacteria should have a dark blue to almost dark green colour, while stain precipitates will always be shades of purple and tend to be variable in size. It is a subtle but well visible difference. Try to compare these colours critically the next time when you are certain that you are looking at a septic smear. Other issue to remember, that cytology is not very sensitive to pick up low numbers of bacteria, thus not seeing intracellular organisms on a slide does not rule out sepsis. Furthermore, if antibiotics have been already used, the odds of seeing bacteria decrease significantly!

Conclusion

Cytology is not just fun, but can be extremely useful in our diagnostic work. Many of the straight forward lesions can be easily diagnosed with good basic training and a quality microscope. Cytology is based on pattern recognition, and contrasting the seen image with pictures of colour atlases are a very valid way of evaluating slides, even on a professional level! The references listed are great resources to have next to our microscopes (previous editions are great as well).

Furthermore, knowing about the limitations of cytology in general, and of our own training level will greatly help in deciding, when to seek second opinion, or choose a different type of diagnostic process (e.g. histopathologic examination). Most well trained pathologist will give detailed descriptions of the slides (not just a conclusion), thus making notes of your own opinion and contrasting that with the report is a great way to continually train yourself, and in fact this doesn't even cost anything to you ☺!

Further reading

Raskin, R; Meyer D. Canine and Feline Cytology: A Color Atlas and Interpretation Guide. Saunders, 2009.

Cowell, RL; Tyler, RD; *at al*. Diagnostic Cytology and Hematology of the Dog and Cat. Mosby, 2008.

Baker, R; Lumsden; JH. Color Atlas of Cytology of the Dog and Cat. Mosby, 1999.

Cytology in Practice

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Advantages of Cytology

- Quick, easy, inexpensive
- Non-invasive
- Minimal risk to patient
- **Screening tool**: determining what diagnostic procedures should be performed next
- Can be useful in establishing a diagnosis or identifying a disease process
  - Certain diagnoses are easy to make!

Limitations of Cytology

- Greatly relies on sample quality
  - Skill of collector
  - Smear quality
  - Type of tissue (exfoliation), site of collection
- Limited by the ability/experience of the person examining the smears
  - Quality of the microscope
- Lack of information about tissue architecture
- Diagnostic challenges of cytology (later)

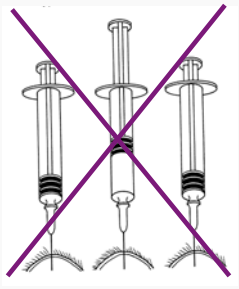
Histopath

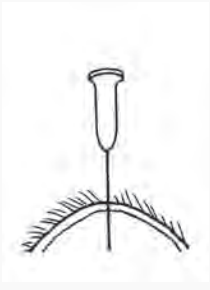
- - More expensive procedure
  - Sterile
- - Slower turn around time
  - 48 hr minimum
- - Poor detail for round cell tumours
- + Tissue architecture
- + Tumour grading
- + Immunohistochemistry more available

Samples for cytology

- **Aspiration or imprints**
  - Superficial masses
  - Lymph node (enlargement, metastasis?)
  - Organs, deep masses (US guidance)
- **Fluids**
  - Body cavities (peritoneum, pleural space, pericardium)
  - Joints
  - Respiratory tract (TTW, BAL)
  - Cerebrospinal fluid

Fine Needle "Aspiration"

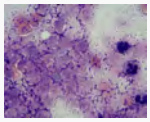




Modified from Cowell at al: Diagnostic Cytology and Hematology of the Dog and Cat, Mosby, 1999

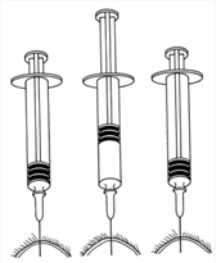
Fine Needle Biopsy

- For solid or fluid filled masses
- Visual or ultrasound guidance
- Similar to FNA but **NO negative pressure** is applied to syringe (no aspiration)
- Use **small gauge** needle (22 -24 gauge)
- Insert into mass several times
- Masses with **necrotic centre** – sample the 'wall' not just the centre
- Then use air filled syringe to expel cells onto slide



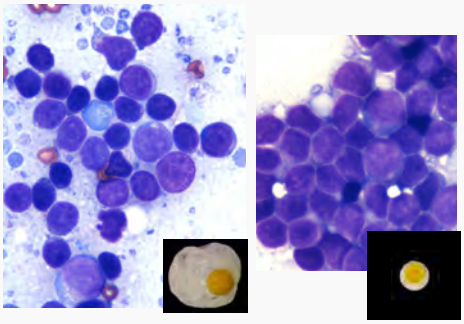
Fine Needle Aspiration

- Only if previous method unrewarding
- With needle in centre of mass, apply negative pressure to the syringe by withdrawing plunger
- Redirect needle 2-3 times to ensure representative sample
- Release plunger (neg. pressure) before removing needle from mass

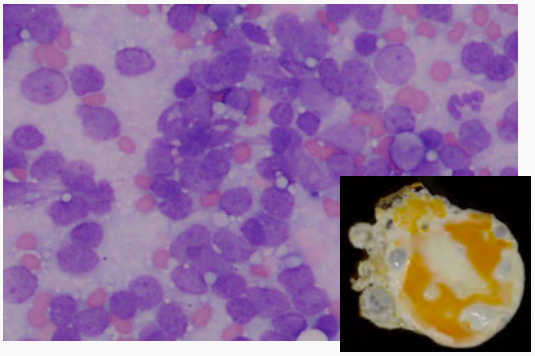


Modified from Cowell et al: Diagnostic Cytology and Hematology of the Dog and Cat, Mosby, 1999

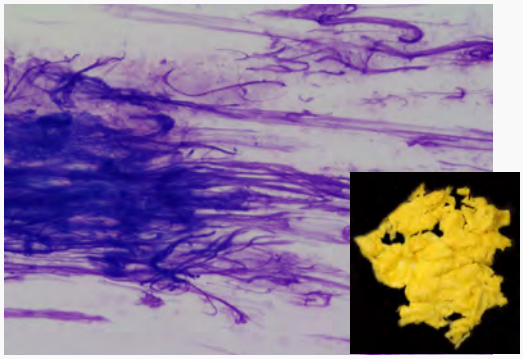
Quality of the Smear



Quality of the Smear

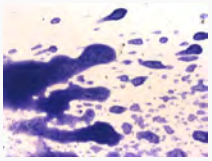


Quality of the Smear



Smear Preparation

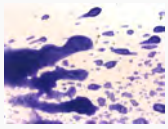
- (Remove needle from syringe)
- Draw air into syringe
- Replace needle
- Expel aspirated fluid onto slide from needle lumen with air-filled syringe
  - Be gentle!
- **Smearing, not spraying!**



Smear preparation

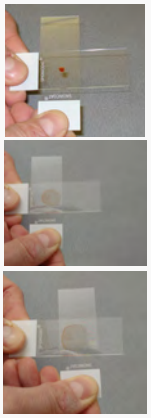
Goals:

- Thin areas with good cell spread
- Minimize cell damage
- Minimize blood content

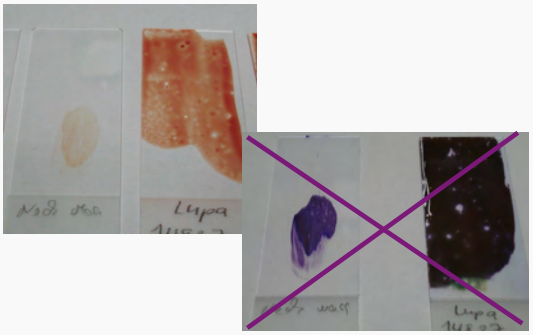


Making Smears

- Put another slide over top – this will spread sample
- Pull slides apart – use gentle pressure
  - If pressure is excessive – rupture cells (especially true of neoplastic cells and lymphocytes)
  - If not enough pressure – too thick

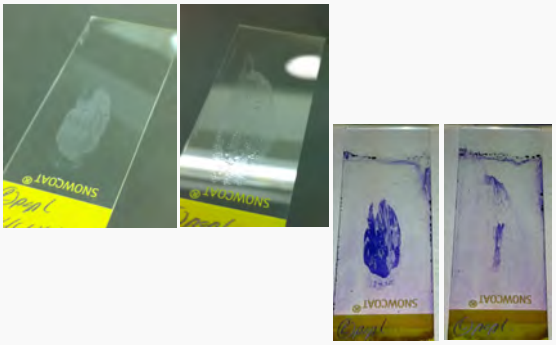


What Should it Look Like?



Blood also contains neutrophils!

What Should it Look Like?

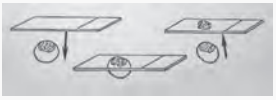


Touch Impression (Imprints)

- Good for evaluation of excised tissue or superficial lesions
- Imprints are made before the excised tissue is placed in 10% buffered formalin and submitted for histopathology

Imprints

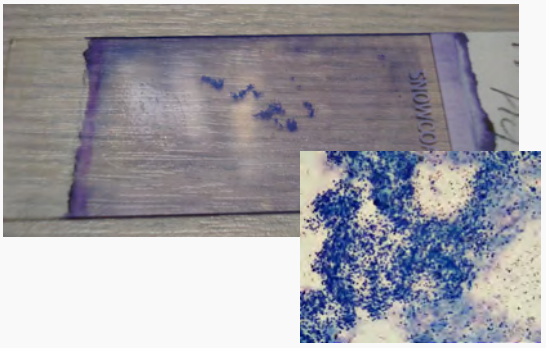
- Use fresh cut surface of the tissue
- Blot until **dry** with paper towel
- Imprint directly onto glass slide
  - Tissue should be rolled against the slide
  - 4-5 imprints per slide
  - Air dry slides and stain



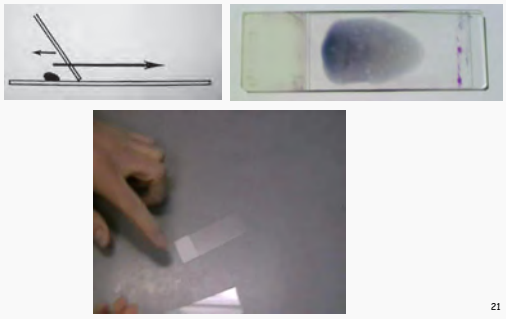
Collection of the Fluid

- Clot prevention – **EDTA!**
- Bacteriology – **Sterile pot!**
- Slide preparations – **Fresh!**
  - Direct smear
  - Line preparation
  - Squash preparation
  - Concentration techniques

Sample Quality – Clots!



Fluids - Direct Smear



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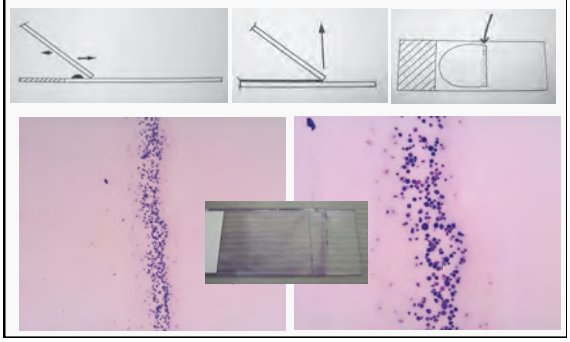
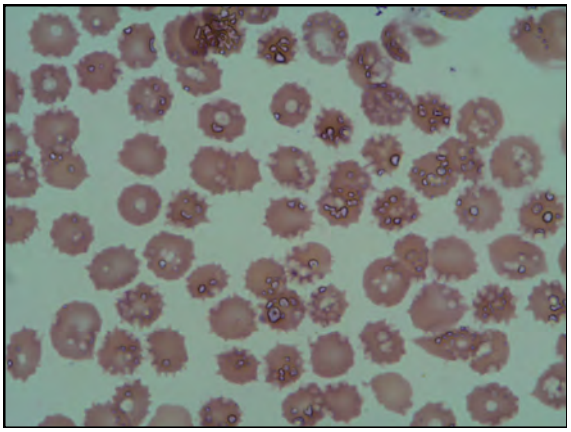
Fluids - Line Preparation



Staining

- **Automated**
- **Manual**
  - Diff-Quik type stains



Diff-Quik "Secrets"

- 1st jar: alcohol – longer the better!
- 2nd jar: red stain – can not overstain!
- 3rd jar: blue stain – number of dips!
  - Can always go back and dip more
  - Blot "mirrory" film from top
- Replace frequently (about 2 weeks)
  - MUST clean jars properly

- \+ Great penetration, nuclear detail
- \- Mast cell granules, basophils
- Use separate set for ear canal, skin

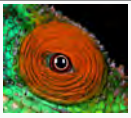
Gram Stain

- Designed for bacterial culture – alive
- Dead bacteria is Gram –
  - Antibiotics
- Nuclear material stains Gram –
- Time of de-staining (false gram + or -)
  - Thick or thin preparation

Objectives: Magnifications

- 4x
  - Great overview of cytology slides and feathered edge of blood smears
- 10x
  - Essential for haematology overview, good in cytology
- 20x
  - Good in cytology and haematology
- 40x
  - "High dry", needs coverslip!
  - Will only give sharp image with the extra layer of glass!!!
  - Drags into oil on slide – need to be careful
- 50x oil
  - Great objective, but expensive
  - Great for leukocyte differential, fantastic in cytology
- 100x oil
  - Essential if 50x oil is not available
  - Highest magnification – best to confirm organisms and fine structures

Eye Pieces: Diameter

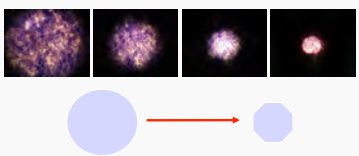


20 mm 22 mm 25 mm 26.5 mm

- Wide angle preferred (22-25 mm is ideal)
- Number of objects (e.g. platelets) per field changes with diameter!
  - Need to modify estimation schemes

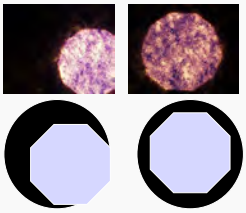
Condenser: How to Set Height

- Köhler illumination
  - Helps to find proper height of condenser
    - 1. Close field diaphragm
    - 2. Elevate the condenser close to top
    - 3. View in eyepiece will change:



How to Centre the Light

- After Köhler illumination is set, start opening the field diaphragm
- Use the two knobs to centre light



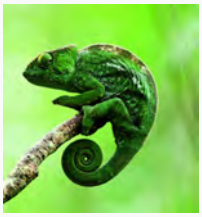
- Open field diaphragm completely

Basic Care and Cleaning

- Remove excess oil from oil objectives with lens paper!
  - Don't use other type of tissues
- If dry objective becomes oily, clean it right away!
  - Will need Q-tip and cleaning solution
- Don't use xylene or similar solvents!

Examination of Smears

- Gross examination
  - Texture (greasy vs. dry)
  - Large cellular clumps
- Microscopic examination
  - Low magnification
  - High magnification



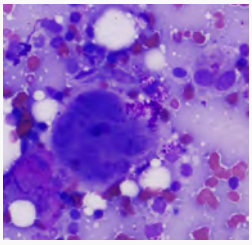
Low Magnification

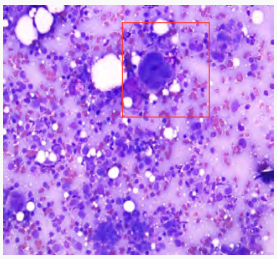
- Cellularity
  - Arrangement – clusters, bundles
  - Shapes
  - Variability
  - Degree of cellularity
- Background – mucus, blood
- Large organisms – larvae, fungi

High Magnification

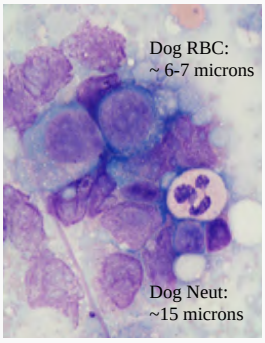
- Evaluate cells populations
  - Inflammatory, epithelial, mesenchymal, round cells
- Evaluate cellular detail
  - Nucleus: chromatin, shapes, number
  - Nucleoli: number, shapes, variability
  - Cytoplasm: colour, amount, inclusions
- Organisms
  - Intracellular or extracellular

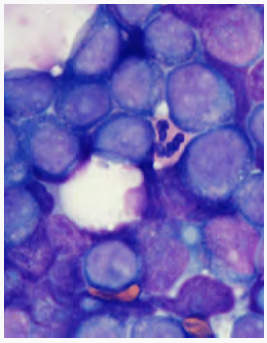
Essential Microscopic Skills



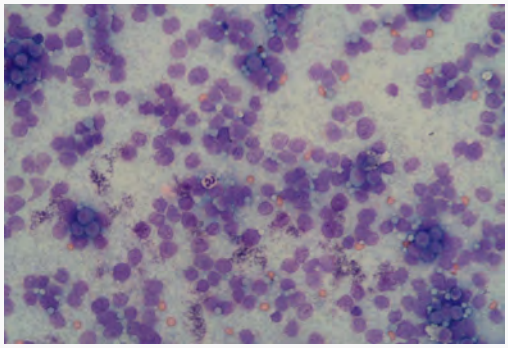


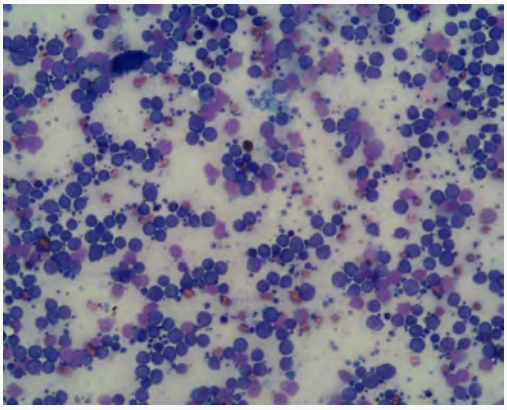
Size Judgement





Finding the Area to Spend Our Time on!

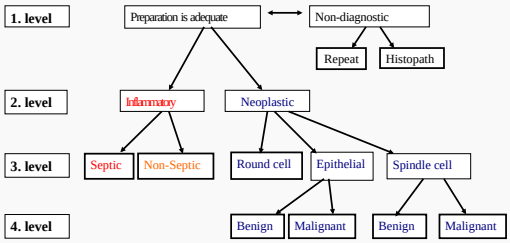




Essential Microscopic Skills

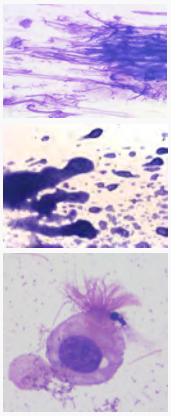
- Your time is expensive – don' t waist it!
- Try to recognize more and more things at lower power
- Use high power to confirm suspicion
- Never lurk around on high power!

Systematic Approach



1. Sample Quality

- Enough cells to examine?
- Preservation of cells
- Adequate spreading
- Is it representative of the lesion
- Do we expect normal cells?
  - Tracheal wash – ciliated cells
  - BAL – alveolar macrophages
  - Joint fluid – mononuclear cells



2. Inflammation vs. Neoplasia

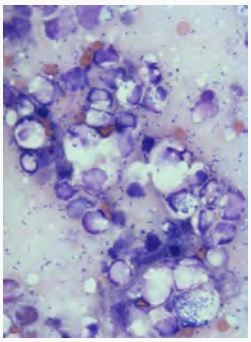
- Sample dominated by inflammatory cells
  - Neutrophils, eosinophils, lymphocytes, macrophages
- Sample dominated by tissue cells → neoplastic
- If both are present – need experience!
  - Inflammation with secondary dysplasia
  - Neoplasia with secondary inflammation

3. Inflammatory: Septic vs. Non-Septic

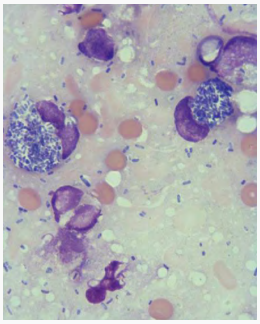
- Septic
  - Contains bacteria/organisms
  - Degenerate neutrophils
  - Bacteria must be intracellular within neutrophils to be significant
  - If extracellular may be contaminants
- Non - septic
  - No bacteria or organisms seen
  - Neutrophils non-degenerate
  - Lack of identifiable bacteria and the presence of non-degenerate neutrophils

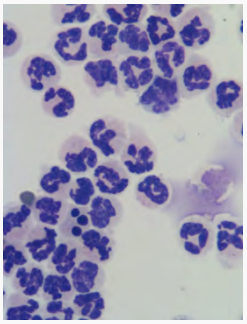
Degenerative Changes in Neutrophils

- Nuclear change
- Nucleus swells, loses lobulation and becomes paler (chromatin becomes less condensed)
- Secondary to release of bacterial toxins
- If degenerate neutrophils are present consider septic cause for the inflammation even if bacteria are not seen

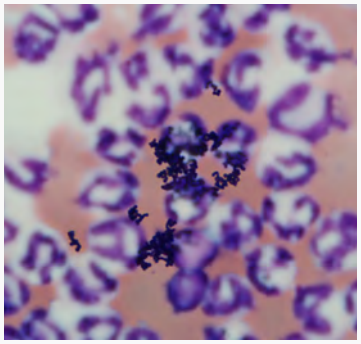


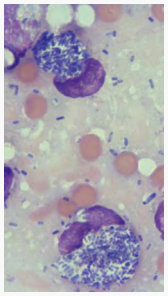
Degenerate vs. Non-Degenerate Neutrophils





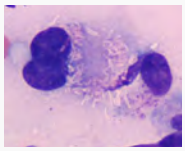
Stain precipitate vs. bacteria

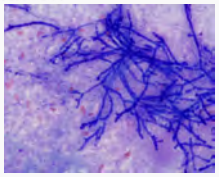




Increased Numbers of Macrophages

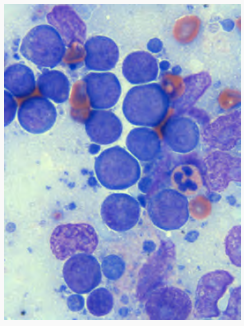
- Granulomatous inflammation
  - Mycobacterium sp
- If neutrophils also – pyogranulomatous inflammation
  - Fungal infections
- Either can occur with foreign body reactions



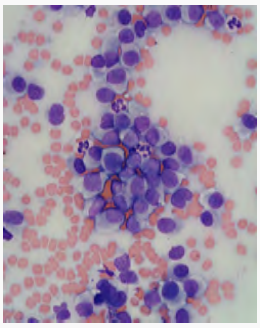
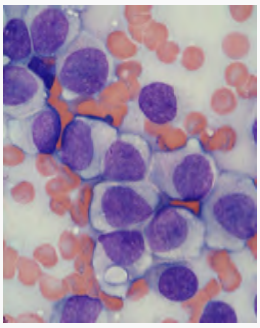


Round Cells

- Individual cells
- Small to medium size
- Round to oval cells
- Round to oval nuclei
- Well defined cell borders
- Good cell yield
- Advantage over histopathology

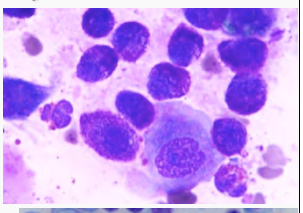
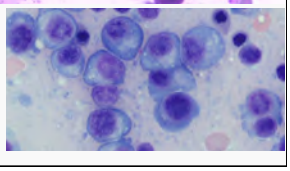


Cell Organization – Beware!

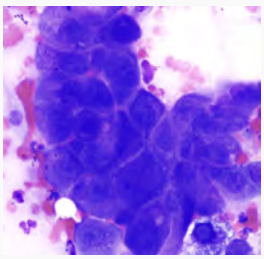
Round (Discrete) Cell Tumours

- Lymphoma
- Histiocytoma
- Mast cell tumor
- Plasmacytoma
- Transmissible venereal tumour (TVT)
- (Melanoma)

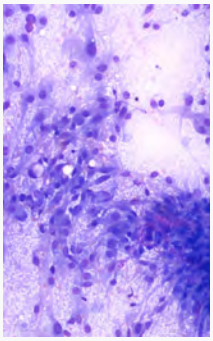
Epithelial Cells

- Often found in sheets/ rafts/clusters
- Large cell size
- Cell-to-cell junctions
- Oval to angular in shape
- Nuclei round, centrally located
- Cytoplasm often abundant
- Good cell yield
- Sebaceous, mammary, liver



Mesenchymal Cells

- Individual cells or clumps
- Small to medium size cells
- Spindle to fusiform to stellate
- Indistinct cell borders
- Elongated nucleus
- Poor exfoliation
- Matrix production - collagen, osteoid/bone



4. Benign vs. Malignant

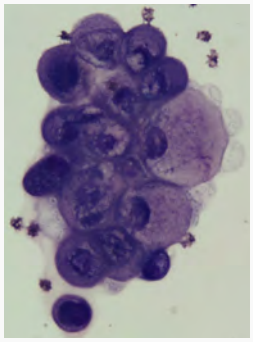
- Epithelial
  - Benign: adenoma
  - Malignant: carcinoma
- Mesenchymal
  - Benign: fibroma
  - Malignant: fibrosarcoma
- (Round cell tumors
  - Biologic behaviour dependent on type)

How to Assess Malignancy

- **Criteria of Malignancy**
- Cytoplasmic and nuclear features associated with malignant behaviour
  - Uniformity vs. pleomorphism (carcinoma, sarcoma)
  - Monotony (lymphoma)
- Nuclear criteria are most reliable
- Need at least 3 nuclear criteria of malignancy before calling a tumour malignant

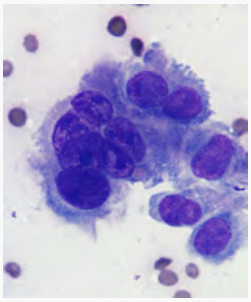
Cellular Criteria of Malignancy

- Anisocytosis - variation in cell size
- Macrocytosis - large cells
- Cell crowding



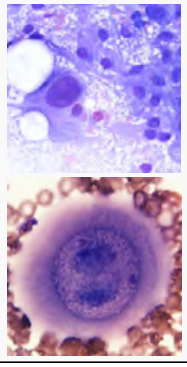
Nuclear Criteria of Malignancy

- Anisokaryosis - variation in nuclear size
- Multinucleation –
  - Nuclei vary in size
  - Odd numbers of nuclei



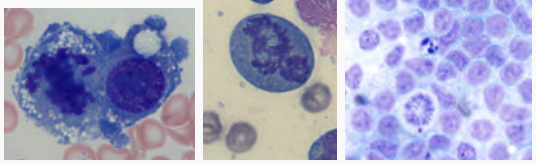
Nuclear Criteria of Malignancy

- Macrokaryosis - giant nuclei
- High nuclear to cytoplasmic (N:C) ratio (in large cells)



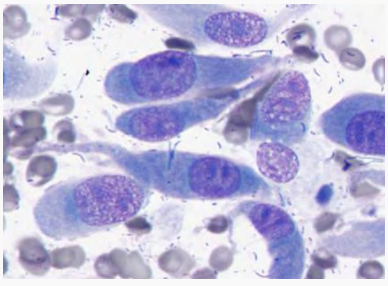
Nuclear Criteria of Malignancy

- Increased mitotic figures
- Abnormal mitotic figures



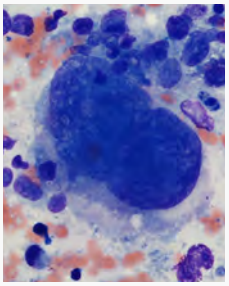
Nuclear Criteria of Malignancy

- Coarse chromatin



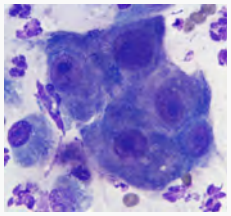
Nuclear Criteria of Malignancy

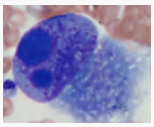
- Nuclear moulding - deformation of nucleus by other nuclei (1 nucleus appears to be growing around another

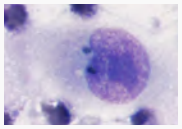


Nuclear Criteria of Malignancy

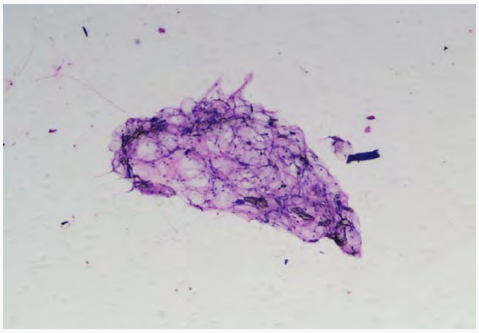
- Macronucleoli - large, prominent nucleoli (RBC)
- Varying nucleolar shape (rectangular, triangular etc)
- Variation in nucleolar size





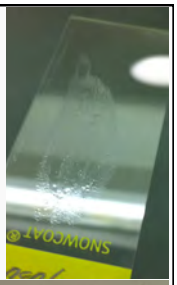


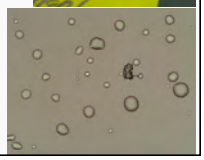
The Important Ones That Doesn't Fit



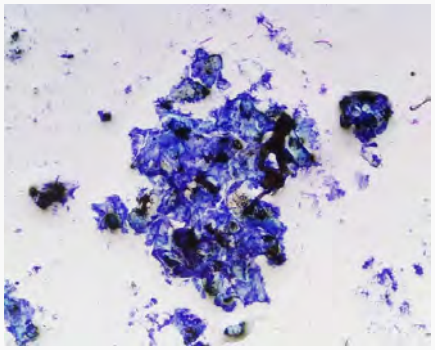
Lipoma

- Most common subcutaneous mass
- Wet appearance before staining
- Subcutaneous fat looks the same





Dermal Mass With "No Cells"



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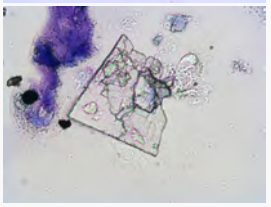
Follicular/ Epidermal Inclusion Cyst

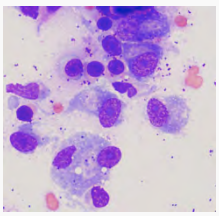
- Common dermal mass
- White chalk like material when dries
- Excision is curative
- Hair follicule tumors

Cholesterol crystals



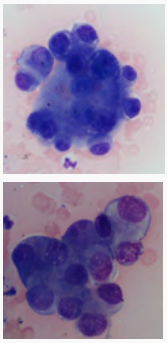
"Unrewarding" Results

- Spindle cells
  - Granulation tissue can have cytologic features of malignancy
  - Well-differentiated sarcoma can look very benign
  - *"Why didn't I just took a biopsy???"*



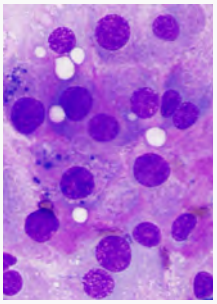
"Unrewarding" Results

- Mammary tumours – mine field
  - Only to differentiate abscess, MCT, lipoma vs. mammary tumour
  - Stromal invasion and metastasis !



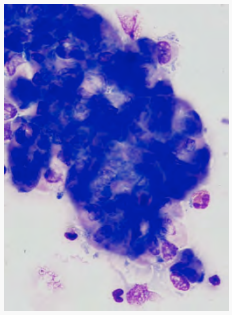
"Unrewarding" Results

- Malignant tumours with uniform cells
  - Thyroid carcinoma - 95%



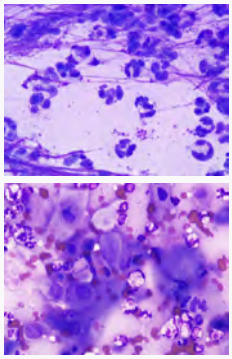
"Unrewarding" Results

- Dysplasia caused by inflammation
- Malignant neoplasia with necrosis, inflammation



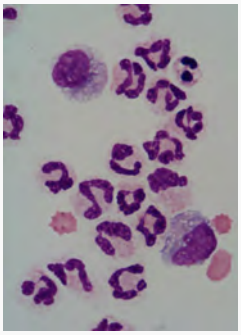
"Unrewarding" Results

- Cytologic diagnosis vs. clinical diagnosis
  - Representative sample



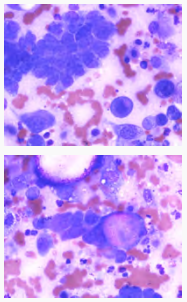
"Unrewarding" Results

- Lack of bacteria does not rule out sepsis
  - Antibiotics
  - Joints



Other Considerations

- Transitional cell carcinoma
  - But how to get the sample?
  - FNA – potential for seeding*
  - Cells mixed with urine
    - Cytopathologist nightmare
    - *Fresh preparation is important!*



*Nyland et at: Needle-tract implantation following us-guided fine-needle aspiration biopsy of transitional cell carcinoma of the bladder, urethra, and prostate; Vet Radiol Ultrasound. 2002 Jan-Feb;43(1):50-3

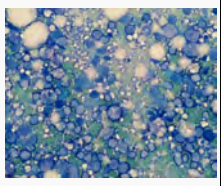
*Vignoli M et al: Needle tract implantation after fine needle aspiration biopsy (FNAB) of transitional cell carcinoma of the urinary bladder and adenocarcinoma of the lung; Schweiz Arch Tierheilkd. 2007 Jul;149(7):314-8

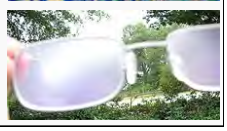
Submitting Samples to the Lab

- Send multiple unstained smears
- LABEL slides/tubes
- Use pencil – will not wash off in fixative/stain
- Signalment (breed, sex, age)
- History, important clinical findings (↑Ca^{2+})
- Describe location of the lesion ("abdominal mass", dermal vs. subcutaneous)
- Duration and rate of growth, previous lesions and diagnosis
- Current therapy (antibiotics, cytotoxic drugs)

Common Problems

- Formalin fumes
- Refrigerating glass slides
- Breakage during shipping
- Lack of freshly made smears (urine!)
- (Flies)





Blind or not blind ?

- **Trained cytopathologist will look at slide objectively**
- **History is critical to formulate comment**
  - Findings are consistent/inconsistent with suspected differential
  - Looking for metastasis – good to know what to look for

Summary

- Microscopic skills
- **Pattern recognition** – experience!
- Use resources (see next slide)
- Some lesions are easy to recognize - lipomas, mast cell tumours, melanoma, follicular cysts
- If in doubt - submit to the lab or biopsy
  - Continued training (paid by the client ☺)

Real Life ☺

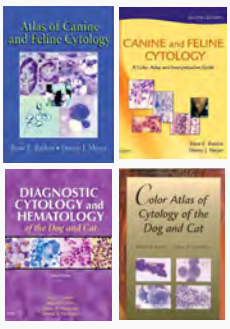
Table 2. Specific diagnoses achieved within category 1

Specific diagnosis	Frequency
Lipoma	105
Mast cell tumour	49
Epidermal inclusion cyst	17
Lymphoid neoplasia	16
Histiocytoma	8
Sebaceous hyperplasia/adenoma	6
Perianal adenoma	5
Anal sac adenocarcinoma	4
Haemangiopericytoma	1
Hepatocellular carcinoma	1
Leishmaniasis	1
Melanoma	1
Multiple myeloma	1
Plasmacytoma	1
Thyroid adenoma (in a horse)	1
Transitional cell carcinoma	1
Total	218

*Skeldon, N and Dewhurst, E: The perceived and actual diagnosticutility of veterinary cytologicalsamplesThe perceived and actual diagnosticutility of veterinary cytologicalsamples; JSAP (2009) 50, 180–185

References

- Atlas of Canine and Feline Cytology – Raskin
- NEW: Canine and Feline Cytology: A Color Atlas and Interpretation Guide – Raskin
- Diagnostic Cytology and Hematology of the Dog and Cat – Cowell
- Color Atlas of Cytology of the Dog and Cat - Baker



SYSTEMATIC APPROACH TO LYMPH NODE ASPIRATES

1. Evaluate the quality of the preparation
    a. Adequate amount of intact cells
        Immature lymphocytes are very fragile, thus many lysed cells are common
    b. Adequate spread
        Thin areas – good aspirates tend to be very cellular, and quite thick!
    c. Adequate staining (thick areas tend to under-stain)
        NEVER INTERPRET UNDER-STAINED AREAS – they always look like lymphoma!
2. Scan the entire slide on low magnification (4X) to find the best areas to spend you time on!
3. Assess the cellular arrangement on low power – look for "foreign" cells (clusters, etc.)
4. Try to decide if it is a uniform population (small matures mostly, or medium to large immature) or a variable population
5. Lymph nodes are "easy", as there is only really 5 big categories in which you have to fit it into based on the proportion (%) of cells types present.

a. Normal

i. Dominated by small, mature lymphocytes (>90%; size of nucleus: **1-1.5 RBC** in diameter, clumped (blocks of) chromatin, small amount of cytoplasm)
ii. Low numbers of medium (size of nucleus: **2-2.5 RBC** in diameter) to large (size of nucleus: **>3 RBC** in diameter), immature lymphocytes (finely granular chromatin, occasionally prominent nucleoli, increased amount of light to deeply basophilic (blue) cytoplasm (<5-10%).
iii. Occasional macrophages, rare neutrophils, eosinophils, mast cells, etc…

b. Hyperplastic or reactive

i. Could be similar populations as normal, but the node is enlarged
    1. If node is enlarged, and the proportions look similar to normal node, by definition it is a hyperplastic node
ii. Increased % of medium to large lymphocyte, but less than 50%!
iii. Possibly increased % of plasma cells (small eccentric nucleus, clumped chromatin, abundant deeply basophilic cytoplasm with prominent perinuclear halo (Golgi zone).
iv. Possibly increased numbers of mitotic figures
v. ALWAYS try to look for reason of hyperplasia/reactivity! (e.g. metastatic tumor, organisms, etc…)

c. Lymphadenitis

i. Increased percentages of inflammatory cells
    1. neutrophils (>5%): 'neutrophilic lymphadenitis'
    2. eosinophils (>3%): 'eosinophilic lymphadenitis'
    3. macrophages: 'histiocytic'/'macrophagic' lymphadenitis
    4. combination of the above, also mast cell % will increase

d. Lymphoma

i. More than 50% immature (medium to large) lymphocytes – *monotony*!
    1. It is not so much the *appearance*, but the *numbers* that count!
ii. Possibly increased numbers of mitotic figures
iii. Only low numbers of small, mature lymphocytes
iv. Plasma cells may or may not be present

e. Metastatic neoplasia

i. Presence of "foreign" cells (even if they don't have ample features of malignancy)
ii. Need to examine all slides
iii. Not finding metastatic cells does not rule it out!

Lymph Node Cytology

Balázs Szladovits - DVM, MRCVS, FHEA, Diplomate ACVP

Pathology and Pathogen Biology

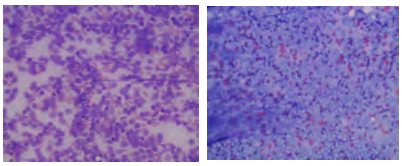
RVC Royal Veterinary College University of London

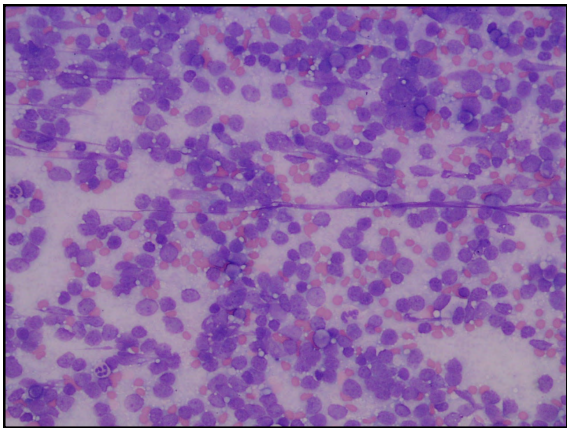
Indications for lymph node sampling

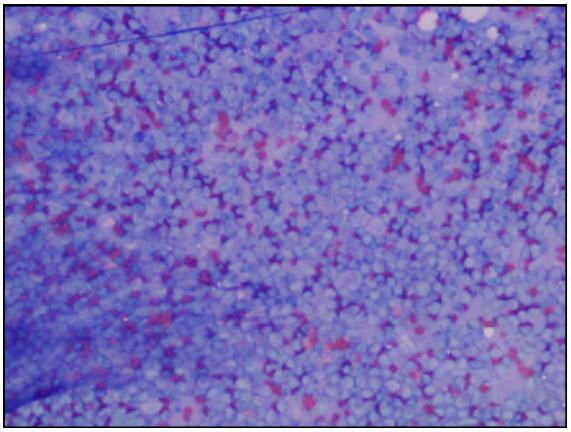
- Lymphadenomegaly
  - Single or multiple nodes
- Evaluation of metastatic disease
  - Based on drainage
    - Submandibular – head (incl. rostral oral cavity)
    - Prescapular – head caudal (pharynx, pinna), thoracic limb, part of thoracic wall
    - Axillary – thoracic wall, deep structures of thoracic limb and neck, thoracic and cranial abdominal mammary glands
    - Superficial inguinal – caudal abdominal and inguinal mammary glands, ventral half of abdominal wall, penis, prepuce, scrotal skin, tail, ventral pelvis, medial part of thigh and stifle
    - Popliteal – distal to stifle
- Classification of lymphoma (?)

Lymph node sampling considerations

- Submandibular vs. popliteal or prescapular
- Aspirating very large nodes
  - Necrotic, hemorrhagic center
- Smearing technique!







Aspirate vs. biopsy

- Invasiveness, cost, turn around time
- Cell detail vs. architecture
  - Lymphoma
  - Metastasis
- Immunocytochemistry

Systematic approach to lymph node aspirates

1. **Evaluate the quality of the preparation:**

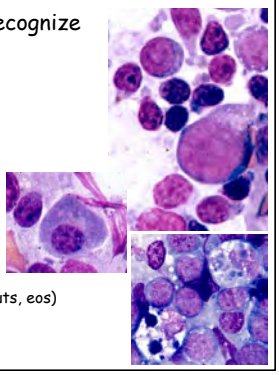
- Adequate amount of intact cells
  - Immature lymphocytes are very fragile, thus many lysed cells are common
- Adequate spread
  - Thin areas – good aspirates tend to be very cellular, and quite thick!
- Adequate staining (thick areas tend to under-stain)
  - ***NEVER INTERPRET UNDER-STAINED AREAS*** – they always look like lymphoma!

Systematic approach to lymph node aspirates

2. **Scan the entire slide on low magnification (4X) to find the best areas to spend you time on!**
3. **Assess the cellular arrangement on low power – look for "foreign" cells (clusters, etc.)**
4. **Try to decide if it is a uniform population (small matures mostly, or medium to large immature) or a variable population**
5. **Lymph nodes are "easy", as there is only really 5 big categories in which you have to fit it into based on the proportion (%) of cells types present.**

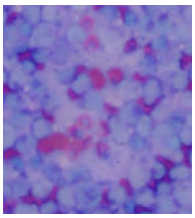
Systematic approach to lymph node aspirates

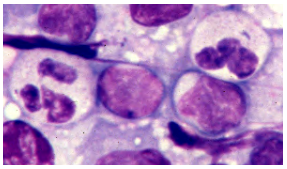
- Only few cell types to recognize
  - Small lymphocyte
    - 1-1.5x RBC
  - Medium lymphocyte
    - 2-2.5x RBC
  - Large lymphocyte
    - >3x RBC
  - Plasma cell
  - Macrophage
  - Few others
    - Inflammatory cells (neuts, eos)
    - Mast cells
    - Foreign cells

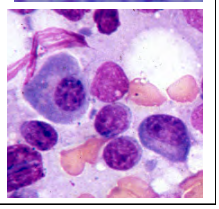


Size judgement

- Adequate spread
- RBCs
- Neutrophils







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- Could be similar populations as normal, but the node is enlarged
  - If node is enlarged, and the proportions look similar to normal node, by definition it is a hyperplastic node
- Increased % of medium to large lymphocyte, but less than 50%!
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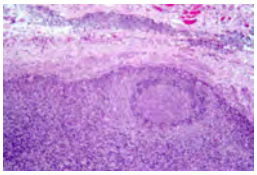
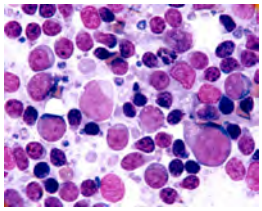
- Possibly increased numbers of mitotic figures
- ALWAYS try to look for reason of hyperplasia/reactivity! (e.g. metastatic tumor, organisms, etc…)

Hyperplastic lymph node

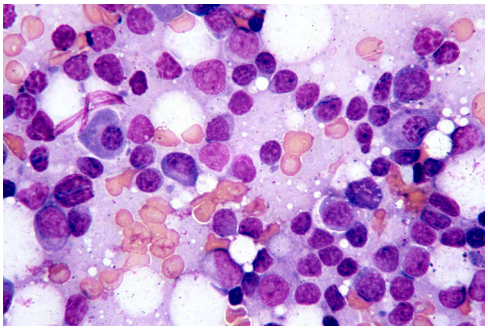


Lymph node section (H & E stain)



Lymph node aspirate (Wright stain)

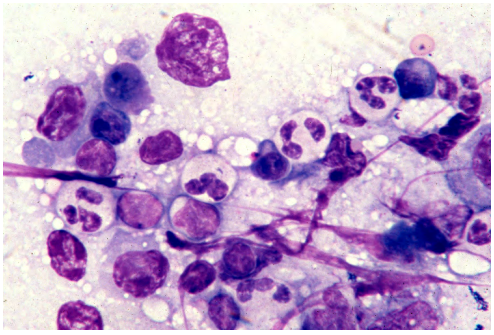
Reactive lymph node



Lymphadenitis

- Increased percentages of inflammatory cells
  - Neutrophils (>5%): 'neutrophilic lymphadenitis'
  - Eosinophils (>3%): 'eosinophilic lymphadenitis'
  - Macrophages: 'histiocytic'/'macrophagic' lymphadenitis
  - Combination of the above, also mast cell % will increase

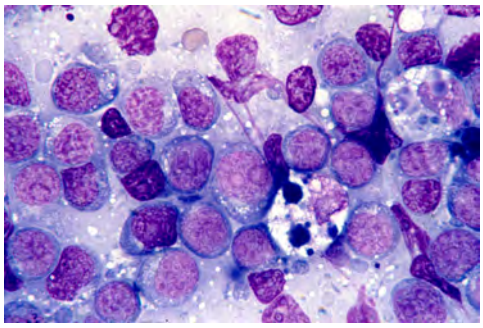
Lymphadenitis



Lymphoid neoplasia

- More than **50%** immature (medium to large) lymphocytes – ***monotony***!
  - It is not so much the ***appearance***, but the ***numbers*** that count!
- Possibly increased numbers of mitotic figures
- Only low numbers of small, mature lymphocytes (rare really large)
- Plasma cells may or may not be present

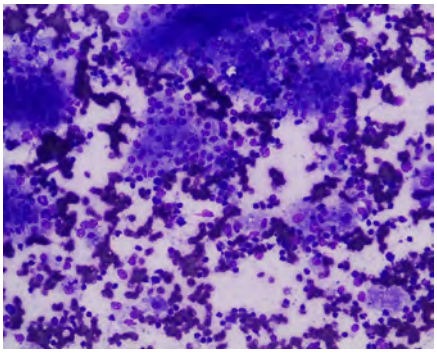
Lymphoid neoplasia



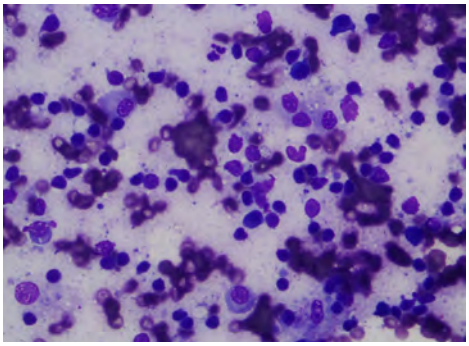
Metastatic neoplasia

- Presence of "foreign" cells (even if they don't have ample features of malignancy)
- Need to examine all slides!
- Not finding metastatic cells does not rule it out!

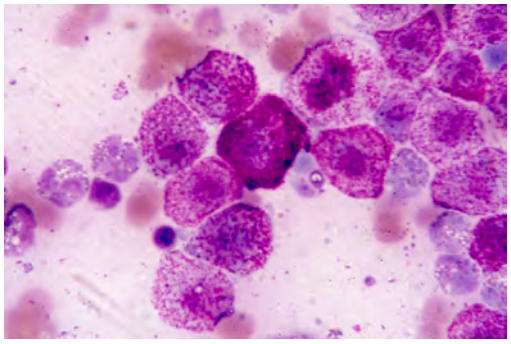
Metastatic neoplasia



Metastatic neoplasia

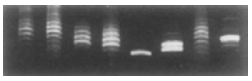


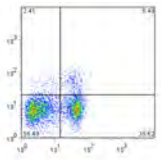
Metastatic neoplasia



New diagnostic techniques

- PCR for T or B cell receptor re-arrangement
  - Clonality
- Flowcytometry
  - Immunophenotyping
- Immunocytochemistry





References

- Canine and Feline Cytology: A Color Atlas and Interpretation Guide – Raskin
- New: Diagnostic Cytology and Hematology of the Dog and Cat – Cowell
- Fundamentals of Veterinary Clinical Pathology (2nd ed) – Stockham and Scott
- Veterinary Comparative Hematopathology - Valli

Cytology Case Discussions

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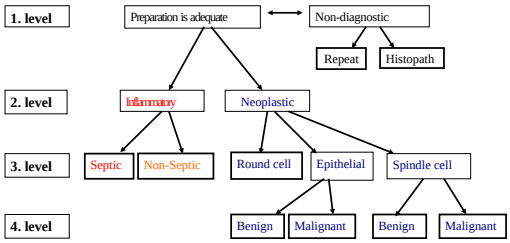
Systematic Approach



Approach to Images

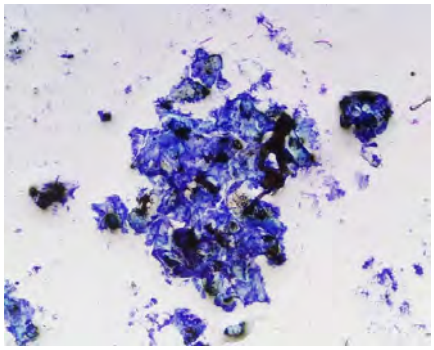
- Low power – judge quality of smear, staining
- Low to mid power – inflammatory or neoplastic
- Higher power – septic vs. non-septic
- Higher power – neoplasia – which category
- Higher power – malignant?

Case 1

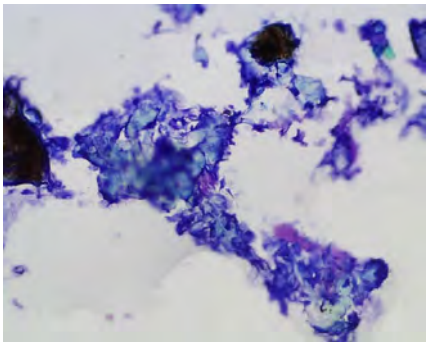
Case history

- 8 year old male neutered mixed breed dog presents to you with a skin mass on his back. The mass has been noticed over the last 4 weeks and has grown considerably during this time. It is now 2x2cm in diameter, firm, and non adhesive to the subcutaneous tissue. You perform a FNA of the mass. The material you aspirated appears like bright white chalk on the slide prior to staining.

Case 1



Case 1



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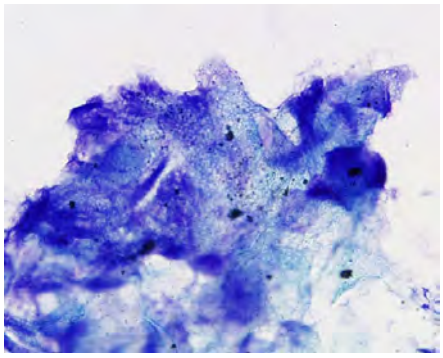

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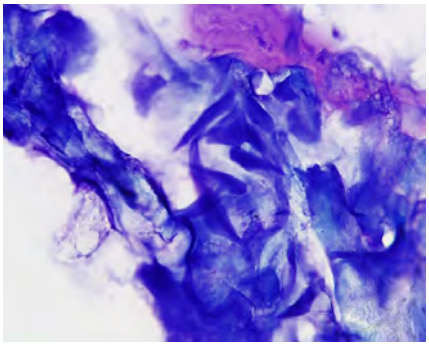

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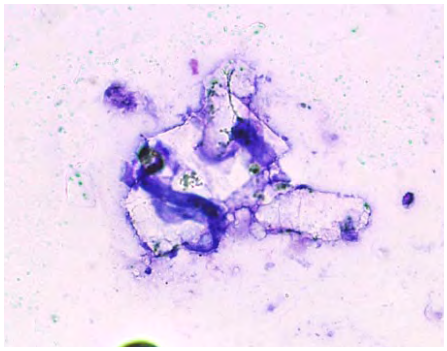


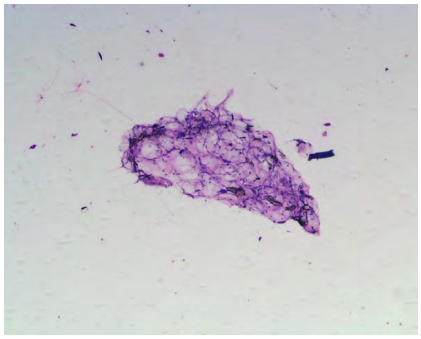
- This is a nice example of a follicular/epidermal inclusion cyst. These lesions commonly contain abundant squames and squamous debris, which appears as a bright white chalk like material on the slide before staining. Squames are anucleated squamous cells, that commonly appear as large, thin, light blue to turquois, angular structures, which tend to fold up, creating fold lines and darker colors where layered. If they roll up completely, we refer to them as keratine bars (Pic 4). Picture 5 contains cholesterol crystals, which are commonly present due to cell (membrane) break down in the middle of the cyst. Note, that benign hair follicule tumors can have similar cystic structures containing the same material, however you would likely to see occasional dense clusters of small, uniform (basaloid) epithelial cells, if the sample is representative, otherwise may only contain the content of the cyst. These cyst tend to grow, as more and more squames are being produced, and ultimately will rupture. As squamous cell debris is very irritative in the dermis, these lesions are best to be removed (both follicular cyst and benign hair follicule tumors). Prognosis is good. The lack of inflammatory cells (neutrophils, macrophages) implies, that this cyst likely hasn't ruptured yet.

Case 2

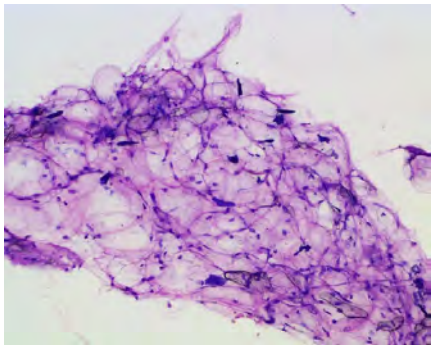
Case history

- 3 year old female spayed mixed breed dog presenting to you because the owner found a lump on her flank. It is 2x3cm in diameter, soft, subcutaneous, non-painful and has grown slowly in the last 3 months.

Case 2



Case 2



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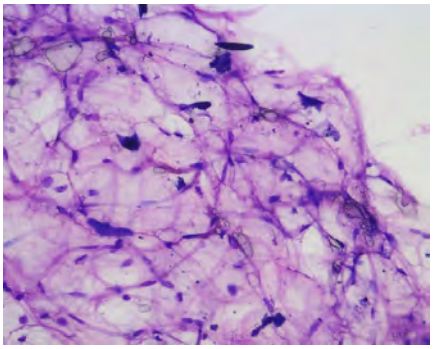
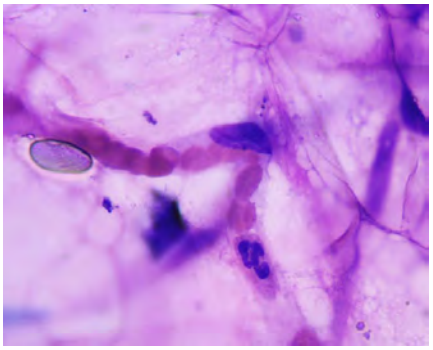


Case 2



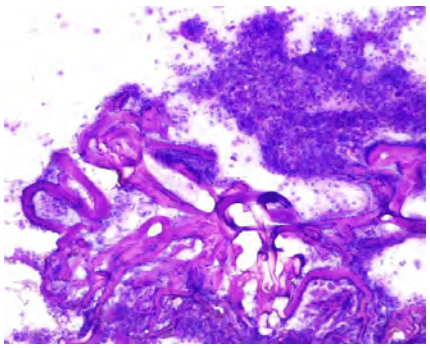
- Consistent with aspiration of adipose tissue (e.g. lipoma or subcutaneous fat). The pictures contain many large adipocytes with abundant cytoplasm containing lipid and small, round nucleus (very low N:C ratio). Many of these slides will appear "wet" prior to staining, but the alcohol fixation will remove the free lipid. Note that it is not possible to tell lipoma cells apart from normal adipose tissue. Many samples will contain aspirated fat, thus to conclude a lipoma, you have to be certain, that what is on the slide, truly represent the lesion you have aspirated, and not just fat around it. Picture 4 is a capillary, and contains several erythrocytes and one neutrophil. You can also see the nucleus of the endothelial cell in the "corner". If you look back on picture three, you can see many capillaries coursing throughout the clump, and the long, thin nuclei of the endothelial cells somewhat mimic spindle cells with tails.

Case 3

Case history

- 3 year old Miniature Schnauzer presenting with an ulcerated subcutaneous nodule on the left 2nd metatarsus which has been noticed over the last 2 months. On physical examination, the nodule is 0.5x0.5cm in diameter and firm.

Case 3



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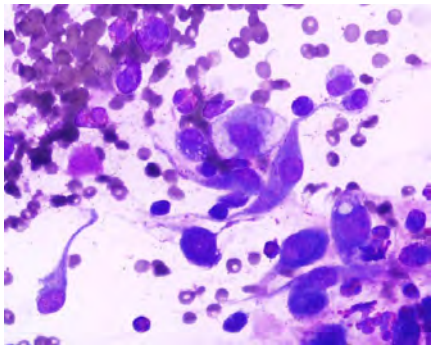

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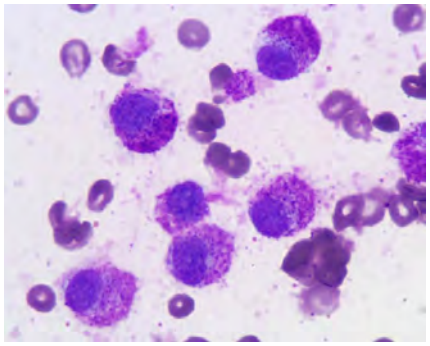
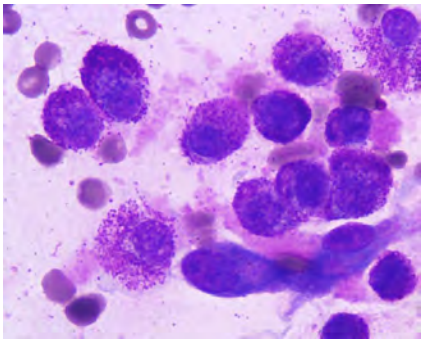


Case 3



- This is a classic example of a mast cell tumor. Most pictures show high nucleated cellularity, low to moderate blood content, and low numbers of lysed cells. Round cells are usually individual, but as seen on Pic 1, it is still common to see large aggregates of cells, if the preparation is thicker. As you can see in the pictures, there is three distinct populations present in this case: mast cells, eosinophils, and plump spindle cells. The ratio of these three will vary from sample to sample. In this case, the mast cells appear well granulated with minimal anisocytosis and anisokaryosis. The abundant, ribbon like material is less common to see, and represent collagen, as mast cells tumors can cause collagenolysis. Lastly, it is very important to emphasize, that cytologic pleomorphism is important in grading, but is only part of the picture, and it is ESSENTIAL to grade via histopathological exam. See the following slides with some key papers regarding grading mast cell tumors.

Case 3

Mitotic Index Is Predictive for Survival for Canine Cutaneous Mast Cell Tumors

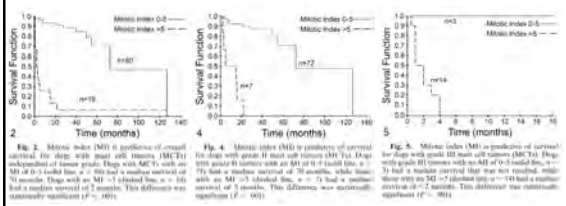
E. M. Romansik, C. M. Reilly, P. H. Kass, P. F. Moore, and C. A. London

Veterinary Medical Teaching Hospital, School of Veterinary Medicine, UC Davis, CA (EMR); Departments of Pathology, Microbiology, and Immunology (CMR, PFM), and Population Health and Reproduction (PHK), School of Veterinary Medicine, UC Davis, CA; and Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH (CAL)

Abstract. Mitotic index (MI) is an indirect measure of cell proliferation that has been demonstrated to be a strong predictor of outcome for several human and canine cancers. The purpose of this study was to evaluate the utility of MI as a predictor of biologic behavior and survival in dogs with cutaneous mast cell tumors (MCTs). Medical records from 148 dogs with histologically confirmed MCTs were reviewed. Information regarding tumor grade, local recurrence, metastatic disease, date of death/last follow-up, and outcome was obtained. The region of the tumor with the highest overall mitotic activity was chosen for evaluation, and the MI value was defined as the number of mitotic figures/10 high-power fields (400×, 2.7 mm²). A Cox proportional hazards regression model was used to compare MI with survival data. A Mann-Whitney test was used to compare MI on the basis of the development of local recurrence and metastatic disease. The MI correlated directly with tumor grade ($P < .0001$). The median survival time for dogs with an MI ≤5 was significantly longer (70 mo[illegible]an for those with an MI >5 (2 months), regardless of grade ($P < .001$). For grade II tumors w[illegible] II ≤5, the median survival time (MST) was 70 months, compared with 5 months for those with an MI >5 ($P < .001$). For grade III tumors with an MI ≤5, the MST was not reached, compared with <2 months for those with an MI >5 ($P < .001$). In conclusion, MI is a strong predictor of overall survival for dogs with cutaneous MCTs and should be included as a prognostic indicator when determining therapeutic options.

Key words: Dog; mast cell tumor; mitotic index.

Case 3



Case 3

Proposal of a 2-Tier Histologic Grading System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior

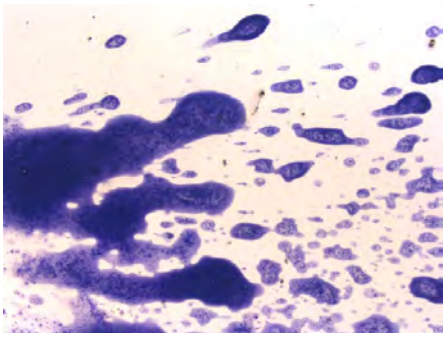
M. Kiupel[1], J. D. Webster[2], K. L. Bailey[3], S. Best[4], J. DeLay[5], C. J. Detrisac[6], S. D. Fitzgerald[7], D. Gamble[7], P. E. Ginn[8], M. H. Goldschmidt[9], M. J. Hendrick[9], E. W. Howerth[10], E. B. Jannovitz[11], I. Langohr[1], S. D. Lenz[12], T. P. Lipscomb[12], M. A. Miller[13], W. Misdorp[14], S. Moroff[1], T. P. Mullaney[1], I. Neyens[15], D. O'Toole[16], J. Ramos-Vara[15], T. J. Sease[15], F. Y. Schulman[11], D. Sledge[1], R. C. Smedley[1], K. Smith[16], P. W. Snyder[12], E. Southorn[4], N. L. Stedman[17], B. A. Steficek[1], P. C. Stromberg[20], V. E. Valli[21], S. E. Weisbrode[20], J. Yager[4], J. Heller[22], and R. Miller[23]

Case 4

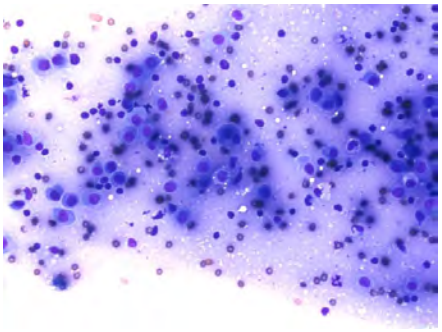
Case history

- 1 year old male intact Golden Retriever presenting to you for a dermal, dome shaped, hairless mass on the top of the nose. The mass is approximately 2x3cm in diameter, firms and moves above the underlying bone.

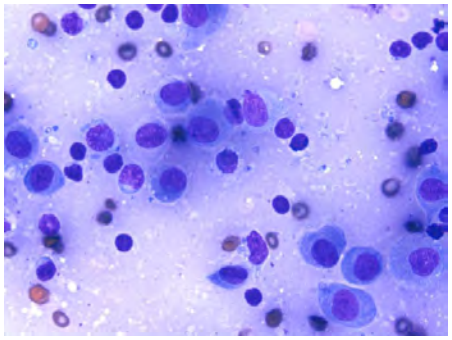
Case 4



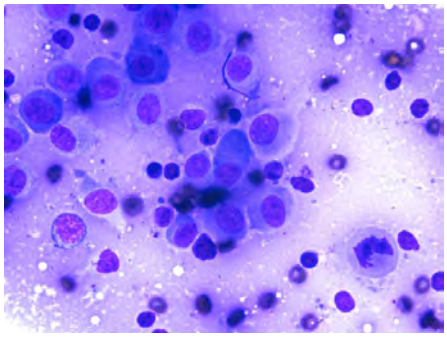
Case 4



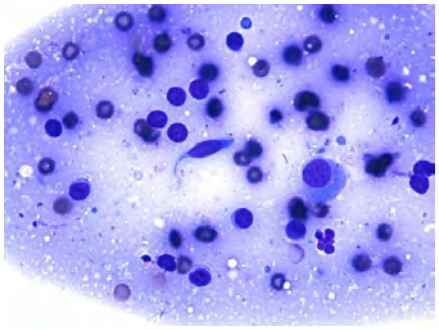
Case 4



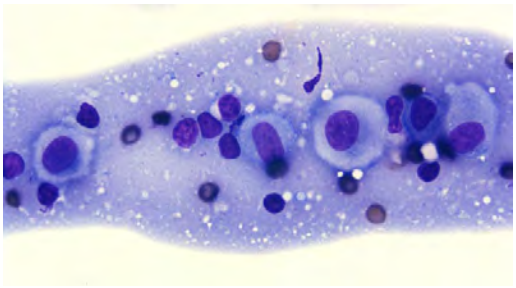
Case 4



Case 4



Case 4



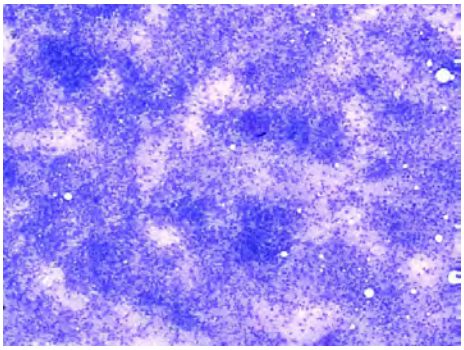
- The preparation had moderate nucleated cellularity. Nucleated cells include many lymphocytes dominated by small, mature lymphocytes, but also low numbers of immature lymphocytes are present. Also there is a moderately large population of round cell with a round central to eccentric nucleus, finely granular chromatin and small nucleoli. The cytoplasm is lightly basophilic. N:C ration is moderate to low. Cellular pleomorphism is minimal.The cytological findings in this case are consistent with a round cell tumor, most likely histiocytoma. It is improtant to note, that occasionally cutaneous plasmacytomas and histiocytomas can have quite similar cytologic picture, however, both are benign as solitary skin lesions. This case is quite interesting, as the dominant cell type in these pictures is the lymphocyte. Histologically, these lesions will have significant infiltration from the deep edge of the tumor with lymphocytes, as the tumor is resolving. Thus likely this is a case of a resolving histiocytoma. Picture 4 has a mitotic figure on the right side, and an immature lymphocyte on the left side. One of the round cells on picture 4 and most of them on picture 6 show the characteristic appearance of histiocytoma cells, as the cytoplasm tends to fade in color toward the rim of the cell. Thus the heavy/thick background in this case helps to highlight this feature. The one spindle cell in picture 5 is likely insignificant, and represent a fibrocyte/blast.

Case 5

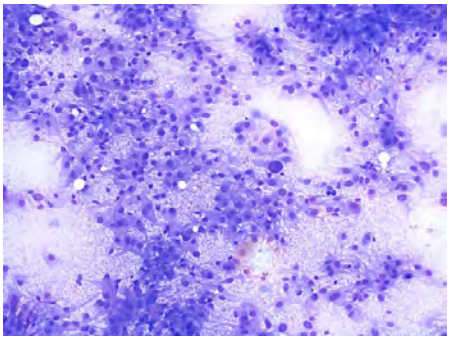
Case history

- 6 year old female spayed Cocker Spaniel which has been treated for 2 months for immune-mediated thrombocytopenia is coming in for a re-examination. The owner noticed a lump between the shoulder blades yesterday and is concerned about this. CBC is normal (platelets are up to normal levels).

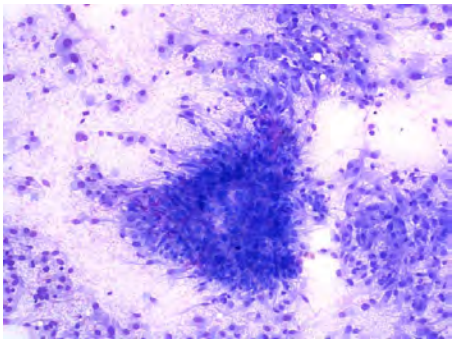
Case 5



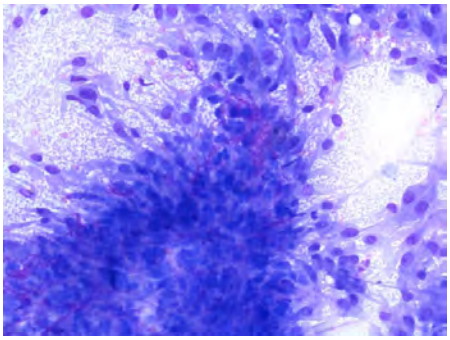
Case 5



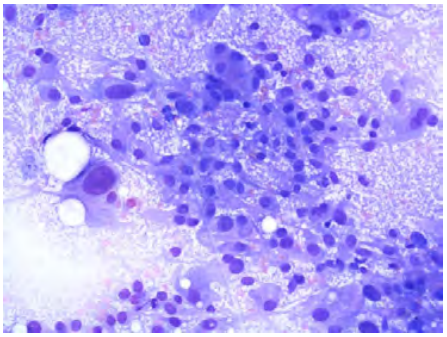
Case 5



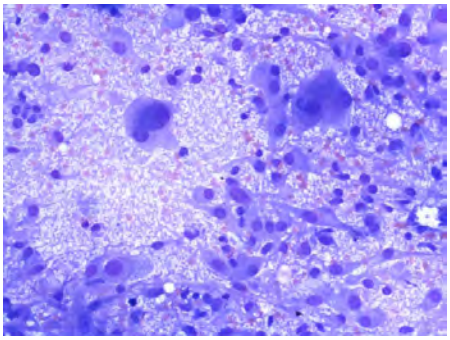
Case 5



Case 5



Case 5



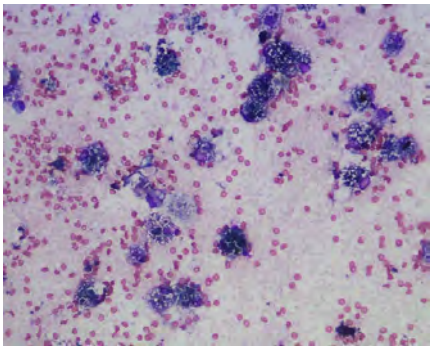
- These pictures showed a preparation with very high nucleated cellularity, few erythrocytes and very good cell preservation. Majority of the cells appear individually, although large clumps of cells are also present, some of which contain pink extracellular matrix material (common in mesenchymal tumors). The dominant cell type is a large population of pleomorphic spindle cells with oval to elongated, variably sized nucleus, prominent nucleoli, and light basophilic cytoplasm with indistinct borders. Features of malignancy included moderate to marked anisocytosis and anisokaryosis, macrokaryosis, multinucleation with anisokaryosis in the same cell, prominent, aberrantly shaped, large, multiple nucleoli (maybe hard to see on the photos, but was easy to see in the scope), and lastly the very high cellularity in face of a mesenchymal type tumor, which generally exfoliate poorly, thus such a high cellularity can also be considered a sign of malignancy. Thus the cytologic diagnosis is malignant mesenchymal/spindle cell tumor. To tell apart which type of soft tissue sarcoma this is, and to establish grade, histopathologic exam is necessary.

Case 6

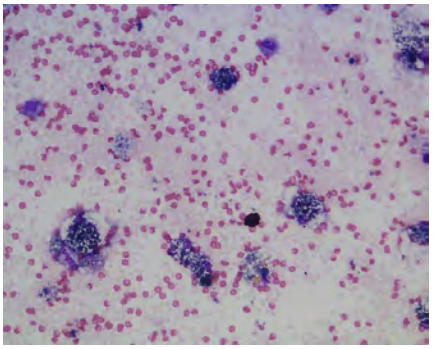
Case history

- 3 months old male Husky presenting to you with multiple subcutaneous masses on the flanks. On physical examination, the masses are soft, fluctuating and subcutaneous, approximately 3x4cm, 3x6 cm and 6x7 cm in diameter. You perform a FNA and aspirate 1-2ml of serosanguineous fluid from each of the masses.

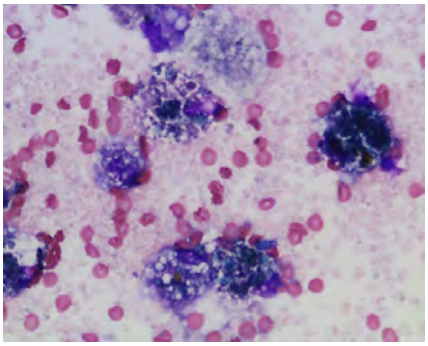
Case 6



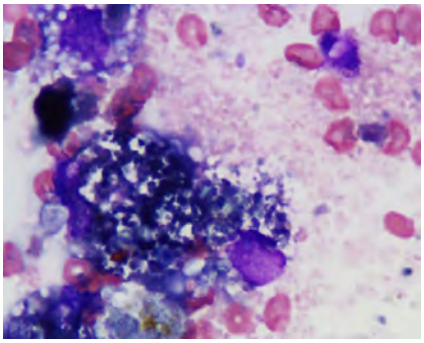
Case 6



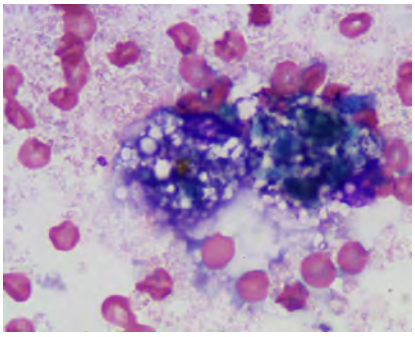
Case 6



Case 6



Case 6



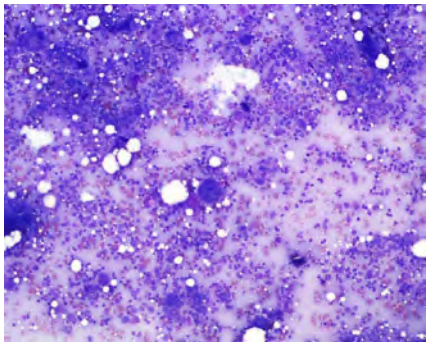
- This is a nice example of a hematoma. The preparation had low to moderate nucleated cellularity, moderate numbers of erythrocytes and abundant debris in the background that was interpreted as erythrocyte ghosts. Nucleated cells were dominated by highly phagocytic macrophages, packed full of hemosiderin (siderophages). Occasional yellow-golden rhomboid crystals were also present, which is called hematoidin. While hemosiderin contains iron, hematoidin is formed from iron-poor hemoglobin pigment, under anaerobic environment. Both structures represent hemoglobin break down, thus indicative of prior hemorrhage. As an exercise, try to go back and see if you can pick up the hemosiderin and hematoidin crystals on the lower magnification images. The clinical diagnosis in this case was congenital hemophilia - once the puppy grew up it didn't bump into things very often anymore and there were no further hematomas diagnosed.

Case 7

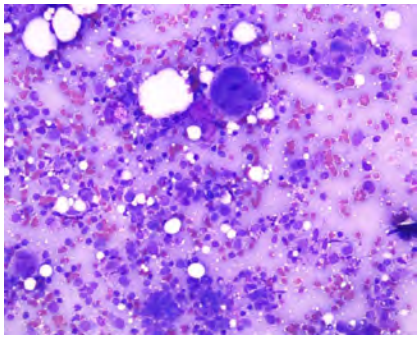
Case history

- 4 year old female neutered DSH cat presenting with a small, firm nodule between the shoulder blades. The owners has noticed the growth appearing 1 week after a vaccination injection against rabies which had been given at this site. On examination, the nodule is subcutaneous, firm, and 0.5x0.3cm in diameter. You perform an FNA.

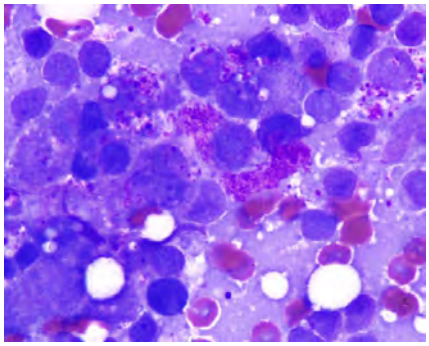
Case 7



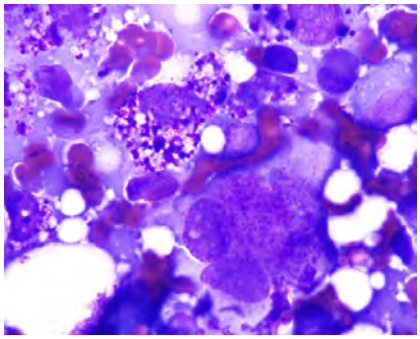
Case 7



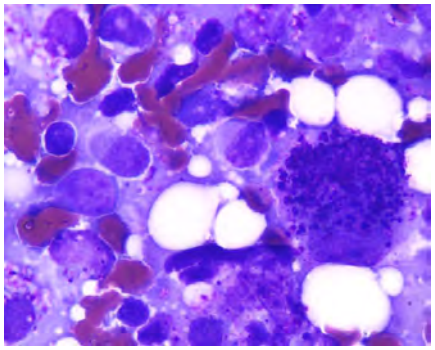
Case 7



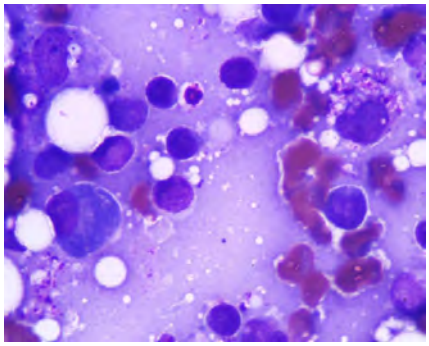
Case 7



Case 7



Case 7



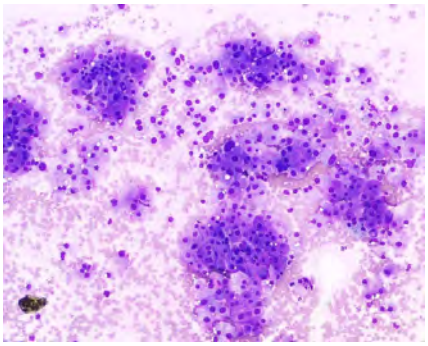
- This is a classic example of an injection/vaccine site reaction. Most pictures show a high nucleated cellularity, moderate numbers of erythrocytes, and low numbers of lysed cells. Nucleated cells are dominated by a large population of lymphocytes, which are mostly small, mature, but there are also low numbers of immature lymphocytes (Pic 5 left), and plasma cells (Pic 6 left). There is also a large population of macrophages, commonly containing abundant red to magenta colored, gobular material (interpreted as adjuvant) (Pic 3,4,5). Multinucleation is common, with low numbers of multinucleated giant cells (foreign body type) (Pic 1,2,4). The background is quite thick and proteinaceous, and also contains the globular red material (in the microscope, it is commonly bright red).
- The lymphocytes (mature, immature and plasma cells) are obviously present due to the antigenic stimulation, and the macrophages are (trying to) "cleaning up" the foreign material. As the macrophages are large cells, they tend to appear more dominant, but it is important not to overlook the smaller cells, such as the mature lymphocytes here. Small cells tend to "hide" in the background, and appear less numerous, as they really are...

Case 8

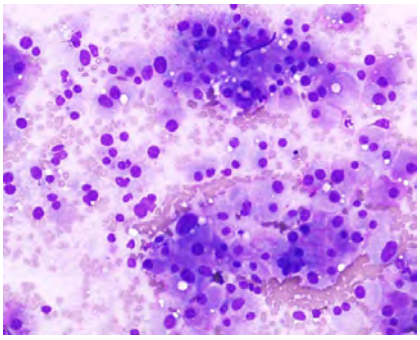
Case history

- 10 year old male intact German Shepherd Dog presenting to you with a mass on the neck which has been growing slowly over the last 2 months. On physical examination, the mass is located subcutaneously below the larynx, soft and fluctuating and 6x8cm in diameter. A FNA is performed under ultrasound guidance.

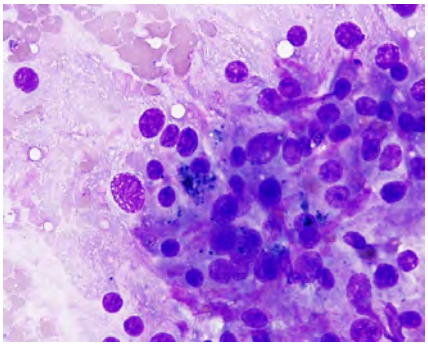
Case 8



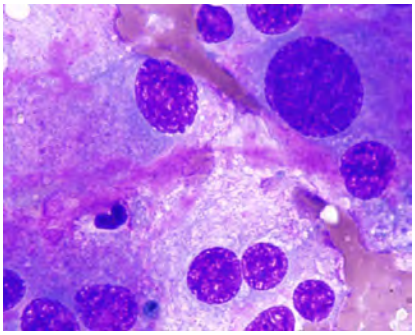
Case 8



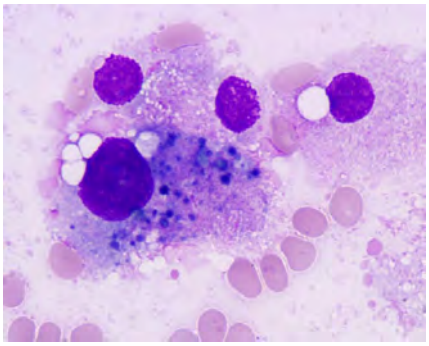
Case 8



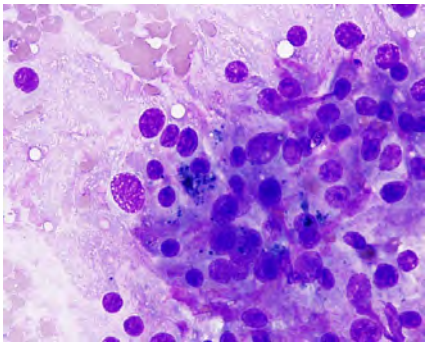
Case 8



Case 8



Case 8



63

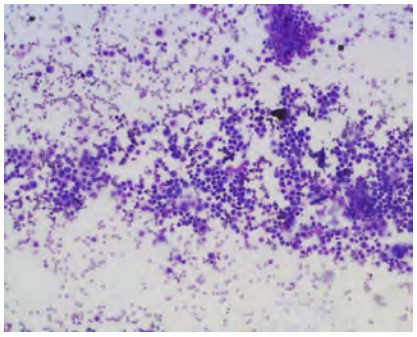
- This is a nice case of thyroid carcinoma. The pictures show a preparation with moderate to high nucleated cellularity, moderate numbers of erythrocytes and many free nuclei in a background of cytoplasmic material. This is commonly referred to as 'endocrine appearance' in cytology. Few of the cells are intact, as they are very fragile, and will commonly lyse during sampling and smearing (even with good technique). The two materials to identify are the pink material between the cells (differentials: extracellular matrix, thyroglobulin, amyloid), which is likely thyroglobulin in this case, and the the dark blue black granular material in the cytoplasm (differentials: tyrosin granules, melanin, hemosiderin), which is tyrosin. Most thyroid carcinomas will appear as a very uniform population of 'free nuclei', but in this case there is considerable anisokaryosis and macrokaryosis. This was actually a functional carcinoma, and both serum concentrations of T_4 an T_3 were increased in this dog. The important question that this case brings up in cytology is the decision of malignancy. In most cases we want to see >3 criteria of malignancy (nuclear) before we can call it malignant cytologically. However, we have to keep in mind that not all malignant tumors will be so pleomorphic. In fact, some of them can be very benign looking on cytology, or even on histopathology, yet very aggressive biologically. Thyroid tumors in the dog are about 95% malignant, regardless of the cytologial picture. So in these cases it is important to recognize that this is a thyroid neoplasm, and you need to have the background knowledge of the biologic behavior in the given species.

Case 9

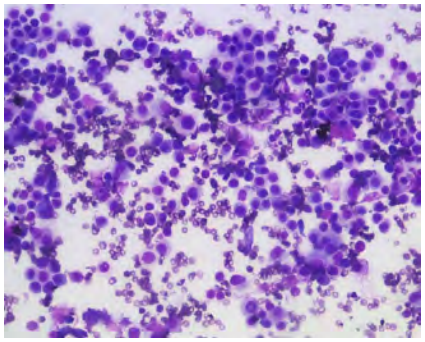
Case history

- 4 year old male intact Golden Retriever presenting to you with a subcutaneous mass near the prepuce. The mass is 2x3cm in diameter and firm.

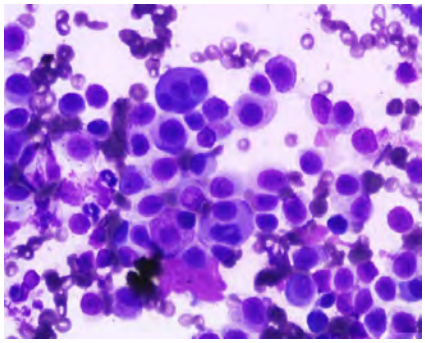
Case 9



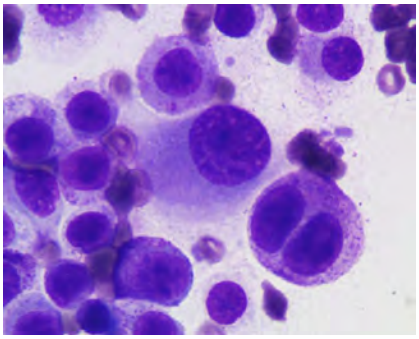
Case 9



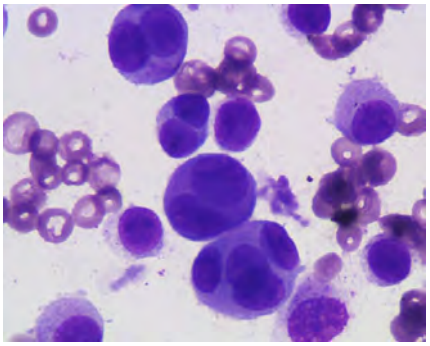
Case 9



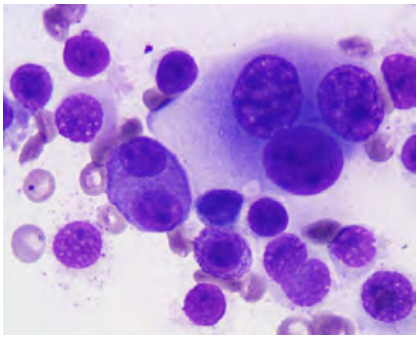
Case 9



Case 9

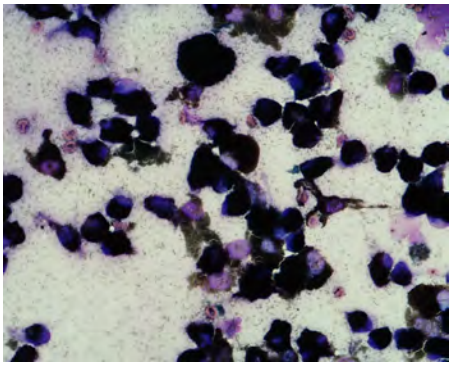


Case 9

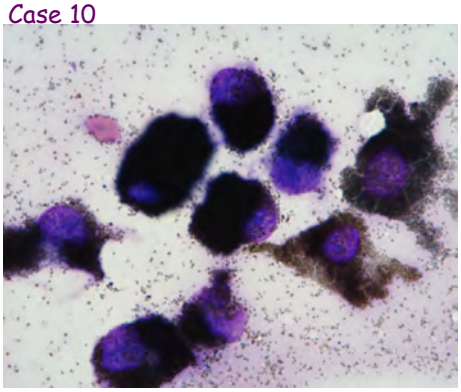


- This is an example of a highly pleomorphic, poorly granulated mast cell tumor. This preparation was stained with Wright Giemsa, thus the lack of granulation in most cells is not a staining artifact, but a legitimate observation. On samples like this, it takes a bit of searching to find a few cells that show some granulation. Please review the images for the marked anisocytosis, anisokaryosis, macrokaryosis, and aberrant multinucleation (seeing odd numbers of nuclei is a strong criterion of malignancy). If no granulation would be found, a plasma cell tumor would also be high on the differential list. Note that the cells without granulation also do resemble histiocytoma cells, but the pleomorphism here would not be expected in such a lesion. Some well granulated mast cell tumors will resemble these images (the lack of granulation), if it was stained with a Diff Quik type stain. In most cases, what could help you is to pick up the presence of eosinophils and the plump spindle cells that were present in the other case previously. However, as biology is highly variable, this sample had hardly any of those "clues"...
- Lastly, it is very important to emphasize again, that cytologic pleomorphism is important in grading, but is only part of the picture, and it is ESSENTIAL to grade via histopathological examination.

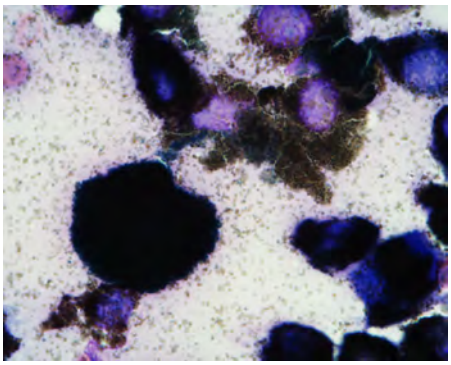
Case 10



Case 10



Case 10



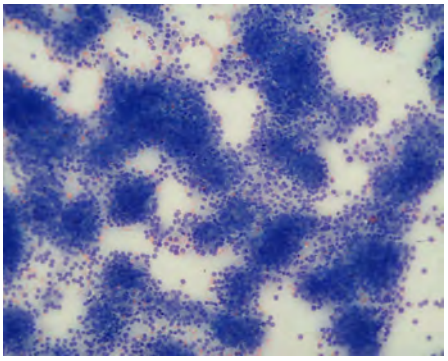
- This is a classic example of melanoma/melanocytoma in the skin. Most of these spindle shaped cells contain abundant brown-black melanin pigment, thus difficult to examine the nuclei for pleomorphism. Also note, that the background also contains abundant free melanin granules. The cell on the left in the last picture could be a melanomacrophage, but difficult to tell. Melanocytes tend to have individualized granules (even if lots), while melanomacrophages will contain more clumped aggregates of melanin.
- With melanoma, the site of the lesion is critical, as e.g. in the mouth or near the digits, it is almost always malignant, while other areas of the body are more likely to be benign. Histopathologic examination is also necessary to establish malignant potential.

Case 11

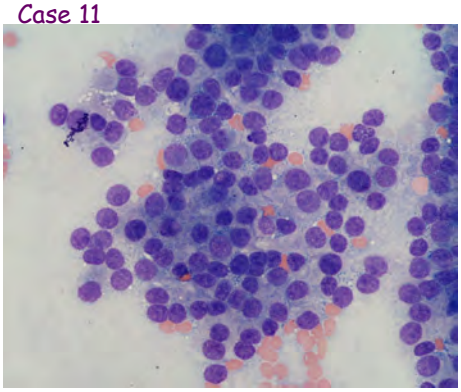
Case history

- A 7-year-old Cocker Spaniel is presented to your practice with a history of PU/PD and tenesmus since 4 weeks. On rectal examination a walnut size mass lesion is palpable on the left hand side, at the level of 8 o'clock.

Case 11



Case 11



- This is a typical example of apocrine gland adenocarcinoma of the anal sac; a very highly malignant tumor, which actually looks more benign cytologically. The pictures show what we refer to as neuroendocrine pattern – many free nuclei in a continuum of cytoplasmic material; this is due to the very fragile nature of the cells, thus they commonly lyse during smearing. The nuclei can look very uniform, however this tumor is invariably very aggressive. Metastasis to the sublumbar lymph node is very common. Many of these dogs will present with the clinical signs (PU/PD) caused by the hypercalcemia – a common paraneoplastic syndrome associated with this malignancy.

Lymph Node Cytology Cases

Balázs Szladovits - DVM, MRCVS, FHEA, Diplomate ACVP

Pathology and Pathogen Biology

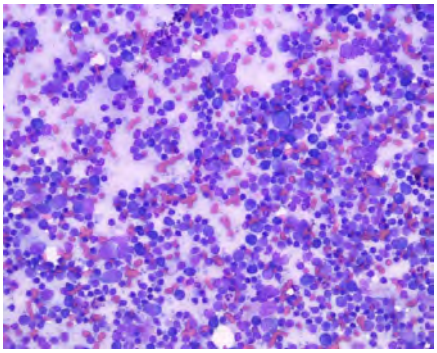
RVC Royal Veterinary College University of London

Lymph nodes – Case 1

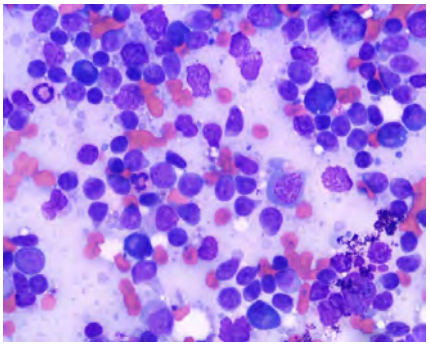
Case history

- 5 year old male neutered Cocker Spaniel with intermittent fever (39.8-41.0 °C) over the last 2 weeks presenting to you with slight generalised peripheral lymphadenomegaly. You perform a fine needle aspirate of the popliteal lymph node.

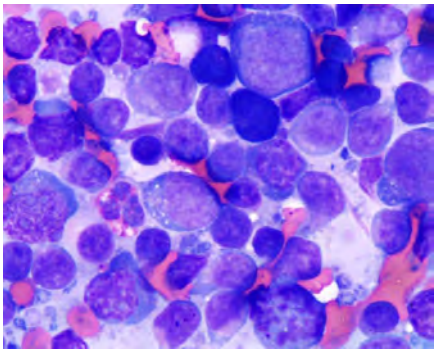
Lymph nodes – Case 1



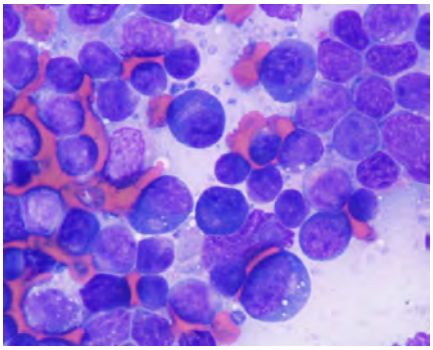
Lymph nodes – Case 1



Lymph nodes – Case 1



Lymph nodes – Case 1



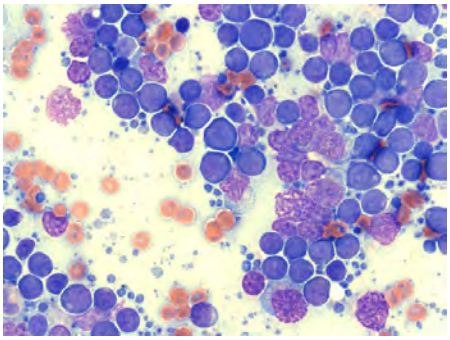
- These pictures show a nice case of reactive lymph node hyperplasia. There is clearly a mixture of different types of lymphocytes including the dominant, small mature lymphocytes with clumped chromatin, no visible nucleoli, and small amount of cytoplasm, but also numerous medium to large, immature lymphocyte with finely granular chromatin, prominent nucleoli, and increased amount of basophilic cytoplasm, occasional mitotic figures (picture 2 top, picture 3 bottom), and many plasma cells with small nucleus with clumped chromatin, abundant, deeply basophilic cytoplasm with a prominent perinuclear clear zone (Golgi zone), and rare neutophils, macrophages, and eosinophils. Try to use erythrocytes as your guide for sizes of nuclei.
- Lymph nodes are the exception in cytology, where "pleomrophism" (variability) is good, and monotony/uniformity is malignant!

Lymph nodes – Case 2

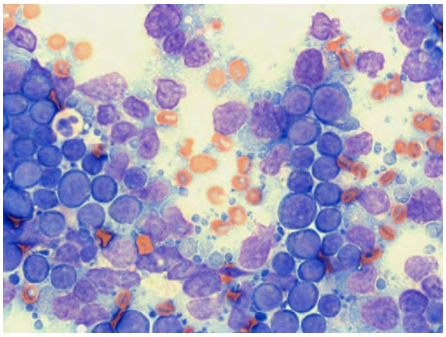
Case history

- 3 year old male intact Boxer presenting to you with PU/PD of 3 week's duration. On physical examination, you notice severely enlarged prescapular and popliteal lymph nodes. You perform FNA's of the lymph nodes.

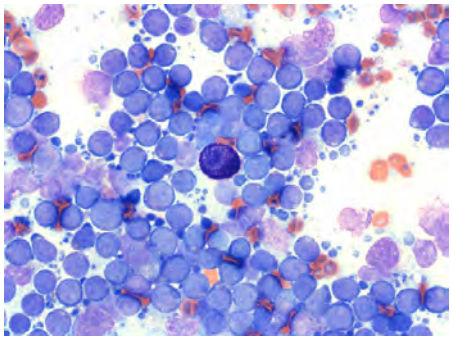
Lymph nodes – Case 2



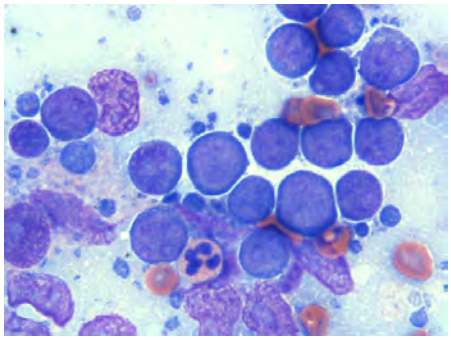
Lymph nodes – Case 2



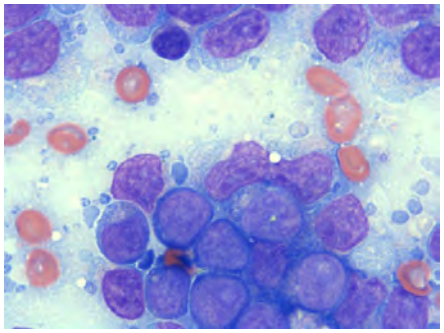
Lymph nodes – Case 2



Lymph nodes – Case 2



Lymph nodes – Case 2



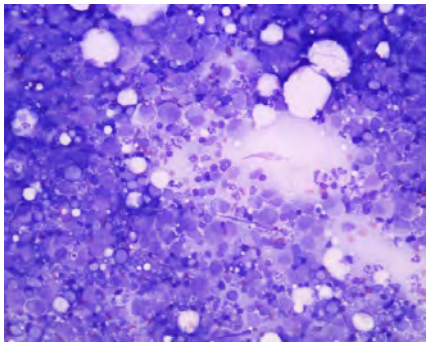
- This a very classic case of a lymphoma. Most images exhibit a very monotonous populations of medium, immature lymphocytes (nuclei is >1.5 RBC in diameter). Their size may differ slightly, but they all have the exact same, finely granular chromatin pattern, prominent, large or multiple nucleoli, and small to moderate amount of deeply basophilic cytoplasm with a small Golgi zone. The last images has a great contrast with the small, mature lymphocyte on the top.
- Note the many large light purple structures – these are lysed cells, which are very common, as neoplastic lymphocytes tend to be fragile, and hence lysed during smearing.

Lymph nodes – Case 3

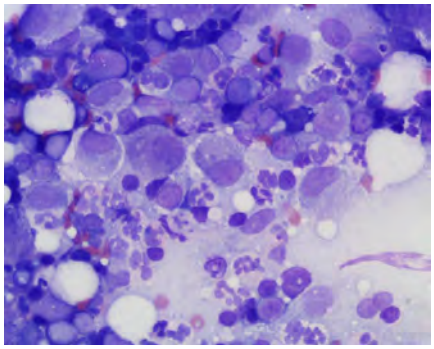
Case history

- 12 year old male intact Flat Coated Retriever presenting to you with sneezing, mucopurulent discharge from both nostrils and epistaxis from the left nostril over the last 2 weeks. Radiographs of the nose show a destructive rhinitis on the left side, suggestive of neoplasia. You perform rhinoscopy and at the same time, take a FNA of the mandibular lymph nodes, as they are enlarged.

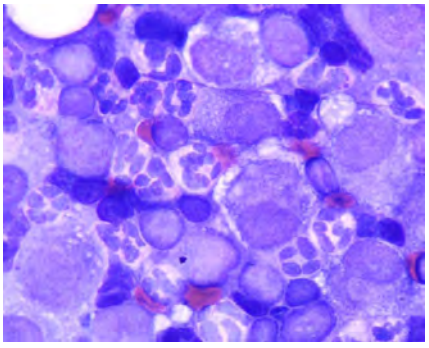
Lymph nodes – Case 3



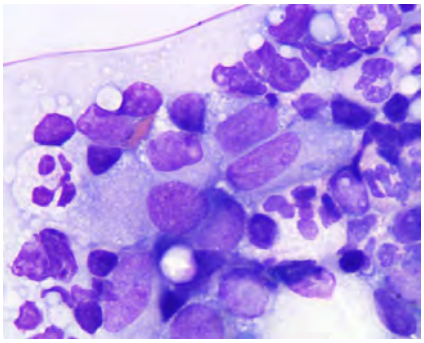
Lymph nodes – Case 3



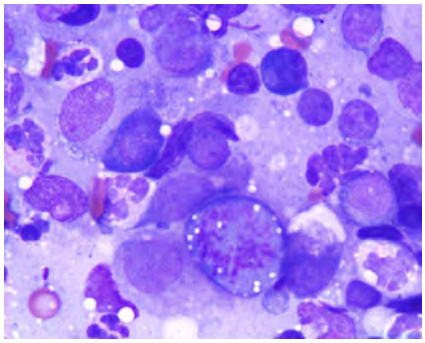
Lymph nodes – Case 3



Lymph nodes – Case 3



Lymph nodes – Case 3



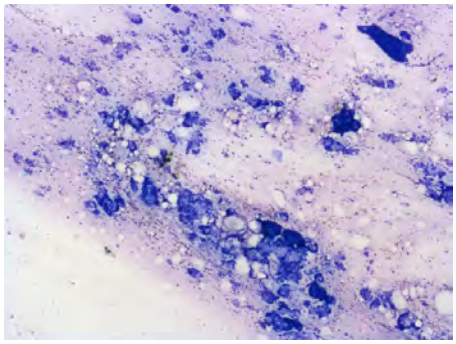
- This was a very interesting case, as we were expecting a nasal tumor, and we were looking for metastasis. However, this is a great case of a marked neutrophilic macrophagic lymphadenitis, which would be most consistent with a pyogranulomatous type lesion.
- The images show numerous neutrophils and large mononuclear cells that are in fact macrophages. There are also numerous small lymphocytes, fewer medium lymphocytes, and occasional plasma cells present, thus we can be quite confident, that a lymph node has been sampled. The structure in the bottom middle of the last image is a mitotic figure. Microorganisms were not seen.

Lymph nodes – Case 4

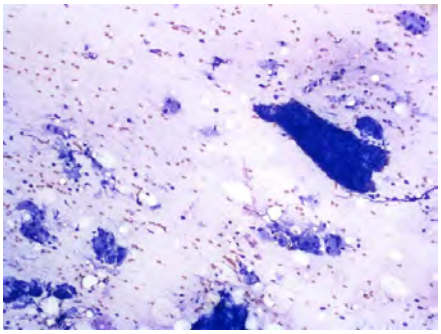
Case history

- 10 year old female neutered West Highland White Terrier presenting to you with lethargy and inappetence. You examine the dog and find that all peripheral lymph nodes are moderately enlarged. You take an FNA of the submandibular lymph nodes.

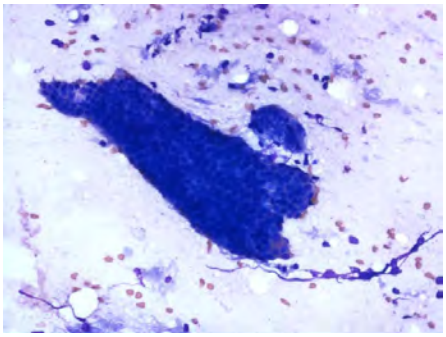
Lymph nodes – Case 4



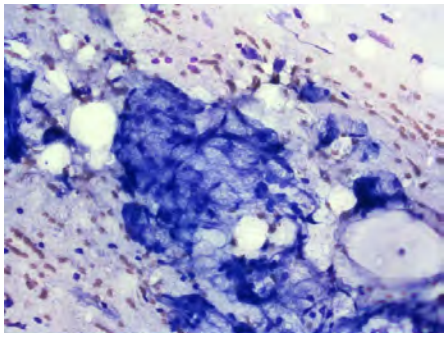
Lymph nodes – Case 4



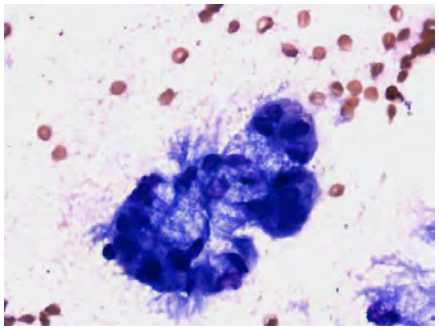
Lymph nodes – Case 4



Lymph nodes – Case 4



Lymph nodes – Case 4



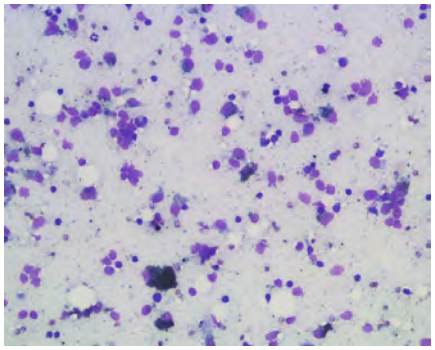
- This a typical case of aspirating salivary gland instead of lymph node. Please note on the very first image the windrowing of the erythrocytes, which suggests a mucinous, viscous background. The large dense sheet of cells is a cluster of epithelial cells, most likely ductal origin. Note how uniform the cells are. The later images show large clusters of epithelial cells, with low N:C ratios, abundant foamy cytoplasm and small, eccentric nucleus. The cells overall are still uniform. These are also salivary epithelial cells, the secretory type. If these cells are individualized, they are often very similar in appearance to phagocytic macrophages. The very last images truly gives an almost three dimensional structure, as the secretory cells are forming small glands...
- Seeing epithelial cells in a "lymph node" could be considered as metastasis, however, these cells do not exhibit any malignant features, and we also don't see any lymphocytes, indicating that a lymph node was sampled.

Lymph nodes – Case 5

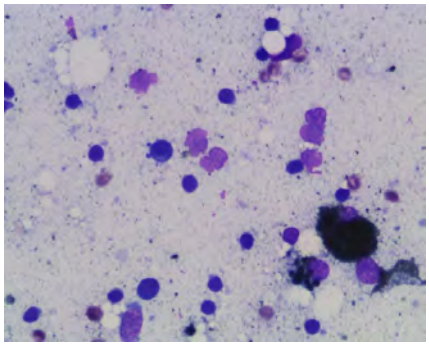
Case history

- 3 year old male intact Boxer presenting to you with an ulcerated oral mass on the left side of the lip. On physical examination, the submandibular lymph nodes are enlarged, and you perform an FNA of the lymph nodes.

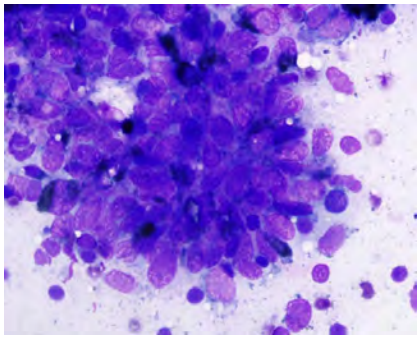
Lymph nodes – Case 5



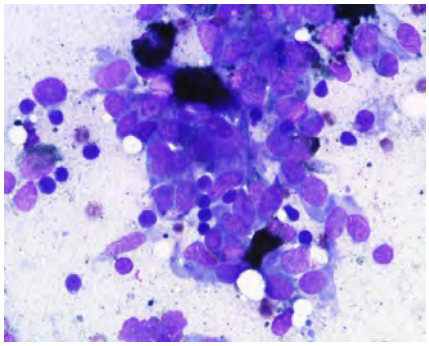
Lymph nodes – Case 5



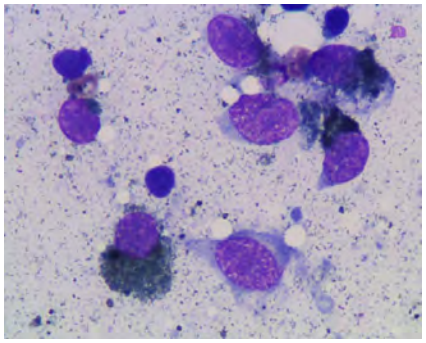
Lymph nodes – Case 5



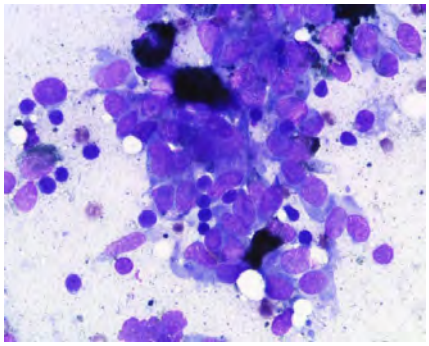
Lymph nodes – Case 5



Lymph nodes – Case 5



Lymph nodes – Case 5



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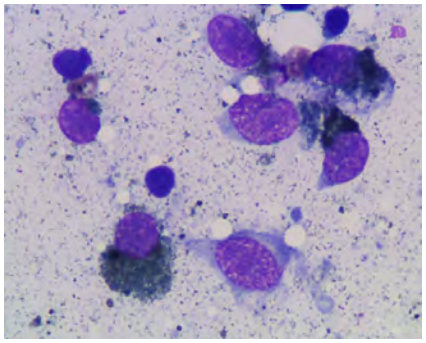
Lymph nodes – Case 5



36

- This sample represent a metastatic malignant melanoma. The first image shows that the cellularity is quite low, with numerous lysed cells/free nuclei. The dominant cell type is still the small lymphocyte, which confirms that we are most likely looking at a lymph node sample, and e.g. not a skin tumor. The large cluster and also the individual cells show marked variability, which is characteristic of malignant melanoma cells, as they can appear almost anything (round, discrete cells, spindle cells, epithelial cells). Here most of them appear spindle shaped with elongated nuclei, have coarse chromatin, multiple or large nucleoli, and lightly basophilic cytoplasm with indistinct borders which contains variable amount of brown green pigment. Occasional cells are heavily laden with the pigment. The latter are more likely to be melanophages (macrophages filled with melanin). The more pleomorphic and less pigmented these cells are, the more challenging it becomes to diagnose it. In fact, amelanotic melanomas typically need considerable experience in cytology to identify.

Lymph nodes – Case 5

Survival of Dogs Following Surgical Excision of Histologically Well-differentiated Melanocytic Neoplasms of the Mucous Membranes of the Lips and Oral Cavity

D. G. Ehrhart

Animal Reference Pathology Division, ARUP Laboratories, Salt Lake City, UT

Abstract. Postsurgical follow-up information was obtained on 64 dogs with 69 histologically well-differentiated melanocytic neoplasms that involved the mucous membranes of the lips and oral cavity. The patients received no adjunct therapy. Sixty one of 64 dogs (95%) were alive at the end of the study or had died of causes unrelated to the tumor, with a mean survival of 23.4 months and a median survival of 34 months after surgery. Twenty-eight dogs alive at the end of the study had a mean survival of 31.3 months after surgery. There were 2 dogs, which had recurrent tumors, that were still alive at the end of the study. All dogs that died of tumor-related causes (3) and all dogs with recurrent tumors (2) had tumors in the oral cavity.

Results of this study indicate that a favorable clinical course and prolonged survival can be expected in most dogs with histologically well-differentiated melanocytic neoplasms of the mucous membranes of the lips and oral cavity, with only local excision of the lesions and no adjunct therapy.

Key words: Canine; lips; melanocytic neoplasms; oral cavity.

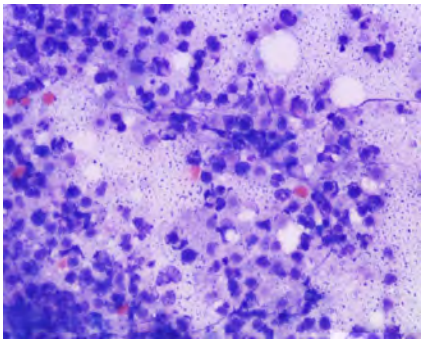
Lymph nodes – Case 6

Case history

- 6 months old male Leonberger presenting to you for anorexia and fever of unknown origin. Previous treatment with iv fluids and amoxicillin-clavulanic acid for 10 days was unsuccessful. On physical examination, the dog is very lethargic, depressed, febrile at 42.2° Celcius, otherwise the physical exam is unremarkable. You perform a CBC, chemistry profile, urinalysis which are normal. On abdominal ultrasound, you detect several grossly enlarged mesenteric lymph nodes with target lesions. FNA's of the lymph nodes are performed under general anaesthesia and under ultrasound guidance. .

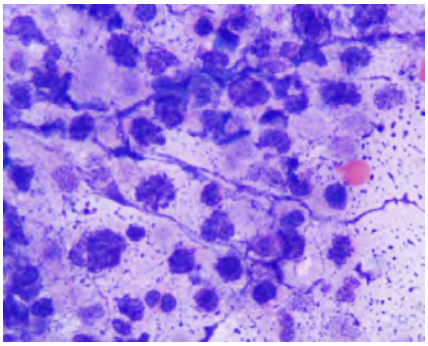
39

Lymph nodes – Case 6



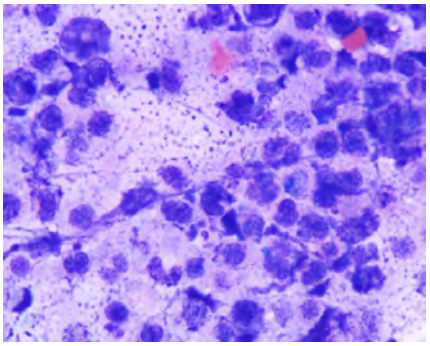
40

Lymph nodes – Case 6



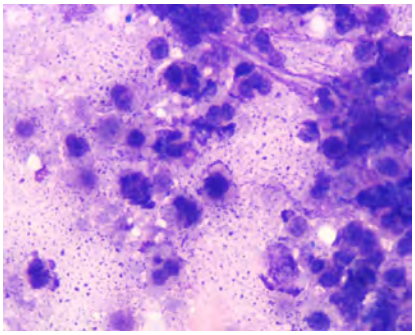
41

Lymph nodes – Case 6



42

Lymph nodes – Case 6



43

- This is a case of severe, necrotizing fibrinosuppurative lymphadenitis (histopathologic diagnosis). The pictures show abundant lysed cells that are just outlines mostly, and there is a very thick, proteinaceous, precipitated background with abundant granular, grey-pink debris. Only very rare neutrophils can be suspected, although many of the cells could have been neutrophils. This is the cytologic picture of necrosis, or when puss is smeared on a glass. Interestingly this case did not grow any bacteria and neither was visible on the slides.

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Foresto ogrlica za mačke i male pse, 1x1,(br. dozvole 365-2010-14-01-001 od 13.10.2011, datum revizije teksta Oktobar 2011.). **Foresto ogrlica za velike pse**, 1x1 (br.dozvole 366-2010-14-01-001 od 24.10.2011., datum revizije teksta oktobar 2011.). **Način izdavanja:** lek se može izdavati samo na recept veterinara. **Indikacije:** tretman i prevencija infestacije buvama tokom 7 do 8 meseci. Kod infestacije krepljima, proizvod ima postojanu akaricidnu i repelentnu efikasnost tokom 8 meseci. Za tretman infestacije pavašima (*Trichodectes canis*). **Kontraindikacije:** Ne koristiti kod mačića mlađih od 10 nedelja i štenadi mlađe od 7 nedelja. Ne koristiti u slučaju poznate preosetljivosti na aktivne supstance ili na bilo koju pomoćnu suspstancu. **Neželjena dejstva:** Kod životinja koje nisu navikle na nošenje ogrlice, u prvih nekoliko dana nakon postavljanja može doći do pojave blagog svraba i/ili eritema. Može doći do pojave neznatnog opadanja dlake i blagih kožnih reakcija na mestu postavljanja, koje obično nestaju u okviru 1 do 2 nedelje, bez potrebnog skidanja ogrlice. U pojedinačnim slučajevima, preporučljivo je privremeno uklanjanje ogrlice do nestanka simptoma. Kod mačaka se, na početku, retko mogu javiti blage i prolazne reakcije kao što su depresija, promene u uzimanju hrane, salivacija, povraćanje i dijareja. Kao i u slučaju bilo kog proizvoda koji se topikalno primenjuje, kod preosetljivih životinja može se javiti alergijski kontaktni dermatitis.
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