Descriptive Cytology

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Descriptive Veterinary Pathology
C.L. Davis DVM Foundation

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Outline

• Introduction
• Techniques
• Interpretation
• Description
• Case examples
Introduction

- Fast
- Cheap
- Minimally invasive
- In-house
- Fun?

![Cartoon Image](https://www.cartoonstock.com/directory/p/pathology.asp)

"I've answered the diagnostic questions 1000's of times..."

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turnaround</td>
<td>Faster</td>
</tr>
<tr>
<td>Cost</td>
<td>Lower</td>
</tr>
<tr>
<td>Detail</td>
<td>Excellent</td>
</tr>
<tr>
<td>Architecture</td>
<td>No</td>
</tr>
</tbody>
</table>

Indications: When Cytology is Great

- Lymphadenopathy
- Bone marrow evaluation
- Blood smear
- Effusions, fluids
- Vaginal smears
- Infectious agents
- Examination for metastasis
Indications: When Cytology is **Okay**

- Cutaneous/subcutaneous mass
  - “Cooperative” neoplasms
    - Easily distinguishable tumors (mast cell tumors)
    - Tumors that exfoliate (round cell, epithelial, PNST)
  - Non-inflammatory / non-neoplastic lesions (sialocele, hematoma, cysts)
- Organomegaly
- Abdominal mass
- Pulmonary, BAL
- Conjunctival/vitreous/aqueous cytology

Indications: When Cytology is **of Limited Value**

- “Uncooperative” poorly exfoliative neoplasms
- Mammary gland hyperplasia/neoplasia
- Some organ aspirates, especially “blind”
  - Spleen
  - Liver
- In a vacuum (must be part of a bigger diagnostic plan)

Architecture!
Outline

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Obtaining Samples

• Fine-needle aspiration/biopsy
• Impression smear
• Swabs
FNA

- 20 or 22 gauge
- 1-1.5 inch needle
- 6 or 12 ml syringe

"Pin-cushion" vs aspiration

Necrosis

Necrosis
'Blood smear' technique

- Fluids
- Lymph node!!
- Non-chunky

Can't tell 'who' these are!
Too dense!

Non-diagnostic

Thicker specimens

Squash prep
**Impression Smears**

- Not best approach

Aggressively debride ulcerated surfaces first; otherwise, true etiology obscured by contaminants

On slide: bits of keratin, blood, +/- inflammation, +/- bacteria

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**Biopsy Touch Impression**

- Perform on biopsy (or autopsy) specimen
- Permits immediate evaluation of a biopsy
- Provides a second means of tissue evaluation
- Valuable instructional tool

- Technique: blot dry on gauze or paper towel until tacky
- Gently blot, don’t wipe, on slide
- Make several, vary pressure

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**Biopsy Impression Smears**

Biopsy specimens – pre-formalin!
Scraping

- Requires cut surface of lesion
- Blot dry and scrape gently with scalpel blade
- Smear onto glass slide
- Useful for mesenchymal tumors

Fluids

- Place immediately into an EDTA tube to prevent clot formation
  - If you just have one drop, make a smear!
- In order to classify most fluids need:
  - Total protein (refractometer)
  - TNCC
  - RBC
  - Cytological evaluation
    - When possible, make direct, centrifuged (buffy coat) and cytospin preps
Helpful Hints

- Good preparation is the key
- Avoid formalin → end up with blue-green cells of 'can't tell' morphology
- Ship separately
- Prepare thin smears (monolayer) & don't apply pressure
- Make smears in a timely manner
- Macrophages ingest things in vitro
- Avoid excess ultrasound gel or lubricant

Staining

- Completely air-dry
- Can use blown air to speed up
- Follow stain instructions

- Diff Quick
  - Great cytoplasmic detail
  - Mast cell granules may not stain well
  - Frequent use → contamination

- Wright Stain
  - Blood smears
  - Complex technique
Trouble shooting

**XS BLUE**
- Too much time in blue solution
- Not enough washing
- Did not let ‘air dry’ enough
- Formalin exposure

**XS PINK**
- Prolonged washing
- Too long in pink solution
- Too short in blue solution

see table 1-3 in C&T, 4th edition

New Methylene Blue

- Excellent nuclear detail
  - Don’t over-interpret nuclear features of malignancy!
- Does not stain erythrocytes
  - Allows identification of reticulocytes and Heinz bodies
- No alcohol fixation = better for lipid-rich samples

Reticulocytes
Coverslip helps

- For sharp focus @ 40X
- Oil on coverslip → 100x
- “Short cut” = dry coverslip on top of slide

No Coverslip / Coverslip
No Coverslip/Coverslip AND Oil!

Outline

- Introduction
- Techniques
- Interpretation
- Description
- Case examples

Interpretation

- Meet your new friends...
ID good samples

- Scan @ low mag
- Cells present? Intact? Preservation? Monolayer?
- Stain ok?
- Assess specimen quality and cellularity

Freeze Artifact

What is this?
Staining

Too little | Just right | Too much

Cellularity & Quality

Most spindle cell tumor cytologies are paucicellular

Cellularity
Stain Contaminant

Bacteria / Yeast

When to suspect contamination:

- Keratinized squamous cells present
- Low #s of bacteria?
- Extracellular location?
- Mixed population?
- Other foreign material or debris present?
- Lack of inflammation?
**Bacterial contaminants**

- **Contamination:**
  - Growing in stain
  - Oropharyngeal
  - From skin/mucosa
  - Inhaled
  - From GI tract

- **Licking**
- **Mouth, respiratory tract**
- **Impression smears**
- **Voided urine**
- **TTW & BAL**
- **Poop**

**Alternaria**

- Common contaminant

*Thanks Dr. Bemis, UT*
Interpretation: Diagnostic Tree

- Mass
  - Inflammatory
    - Neutrophils
    - Eosinophils
    - Macs/Mixed
      - Healthy
      - Degenerate
  - Non-inflammatory
    - Neoplastic
      - Cyst
      - Hematoma
      - Sialocele
      - Mixed
    - Non-neoplastic

Inflammation

- Various species
- Purulent infection
- Abscess
- Fungi?
- Chronic infection
- Immune mediated
- Foreign body
- Resolving infection
- Histoplasma
- Blastomyces
- Sphaerophorus
- Pythium
- Aspergillus

Bacterial infection; moderate neutrophilic inflammation

Which cells predominate? Look for etiology?
Moderate, mixed, predominately eosinophilic inflammation

- If 'many' (>10%)?
  - WORMS
  - WHEEZES
  - WEIRD DISEASES

SQ mass, right hip, canine.

- Mixed Inflammation
- lymphs predominate
- Magenta, globular material

"Blue" vaccine reactions
Description Tips

- Describe bugs that you see! Like histo?
- Paint a picture! If I read your description, should be able to guess the organism...

  - Color?
  - Size?
  - Cell wall?
  - Intra and/or extra-cellular?
  - In what cell types?
  - Character of cytoplasm?
  - Nuclear shape?
  - Nuclear location? color?

RBC (7 μm) & Smaller
- Histoplasma
- Toxoplasma
- Leishmania
- Trypanosoma
- Malassezia
- Sporothrix

Neutrophil (15 μm) & Up
- Cryptococcus
- Blastomyces
- Rhinosporidium
- Coccioidoides
- Hyphae
  • Aspergillus
  • Mucor
- Parasites
Foot lesion aspirate

*Blastomyces dermatitidis*
- royal blue
- thin cell wall
- budding
- estimate size
- round
- inside MNGC

Marked pyrogranulomatous inflammation

*Sporothrix schenckii*

Moderate pyrogranulomatous inflammation
- within in macs
- 2-3 um
- round to cigar shaped
- thin, clear, cell wall
- eccentric, purple nucleus
- light blue cytoplasm

Concentrated prep of abdominal effusion

low-grade macrophage inflammation with intracellular yeast

*Histoplasmosis*
- thin clear wall
- eccentric, comma-shaped, purple nucleus
- light blue cytoplasm
- round to oval
- 1/4 size RBC
**Cryptococcus neoformans, C. gattii**

- Round
- Pink to blue-purple
- 4 to 20 μm in diameter
- Very thick, negatively-staining capsule
- Narrow-based budding
- Typically extracellular; may be phagocytosed by macrophages

**Leishmania**

- 2-4 microns
- Intracellular
- Multiple within macrophages
- Few scattered extracellularly
- Dark purple, ovoid, eccentric nucleus
- <1 μm rod-shaped kinetoplast
- Faint blue cytoplasm

**Fungal Hyphae**

- Extracellular septations
- Thin, clear wall
- Size, shape, branching?
- Surrounded by inflammatory cells
- Marked neutrophilic inflammation

**Aspergillus sp., dog nose**

- 20.0 μm

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Prototheca

Round to oval
5 µm by 20 µm
Blue-purple
thin, clear cell wall
+/- 2-4 small endospores
empty, clear organism casings
extracellular

Toxoplasma / Neospora / Sarcocystis

Tachyzoites in a macrophage
• crescent shaped
• intra/extra cellular
• basophilic cytoplasm
• central purple ovoid nucleus
• 5-7 um

Bordatella bronchiseptica

Neutrophilic inflammation.
(Macrophages live in resp. tract.)

Tracheal wash from a dog
Epithelial cells

White circles: inclusions suspicious for Chlamydomphila felis; often adjacent the nucleus. PCR +

Neutrophils also present

Mycobacterium sp.

VCP 2005; 34(2):161-163

Squamous lesion from a dog

Wright's stain

Acid-fast stain

Diagnostic Tree

Mass

Inflammatory

Neutrophils

Healthy

Degenerate

Eosinophils

Mixed

Non-inflamatory

Non-neoplastic

Neoplasia

Hyperplasia

Cyst

Round

Hematoma

Epithelial

Sialocele

Mesenchymal
Non-inflammatory / Non-neoplastic Lesions

Usually (not always) few to no inflammatory cells:
- Cyst
- Seroma/Hygroma
- Hematoma
- Sialocele
- Neoplasm

Abundant amorphous keratinized debris
few cholesterol crystals

Keratin producing cyst or neoplasm

Infundibular keratinizing acanthoma

Cholesterol crystals
LIPOMA or SUBCUTANEOUS FAT

Platelets indicate blood contamination, rather than hematoma (or hemoabdomen/hemothorax, etc).

Hematoma vs. Hemorrhage

Platelets indicate blood contamination, rather than hematoma (or hemoabdomen/hemothorax, etc).
Hematoma vs. Hemorrhage

- Erythrophagocytosis + hemosiderin pigment

Sialocele

- Streams of blue mucinous material
- Rowing of erythrocytes
- Hematoidin crystals

Cholestasis
Diagnostic Tree

Mass
  - Inflammatory
    - Neutrophils
      - Healthy
      - Degenerate
    - Eosinophils
    - Mixed
  - Non-inflammatory
    - Neoplastic
      - Neoplasia
      - Hyperplasia
    - Cyst
    - Hematoma
    - Epithelial
    - Mesenchymal

Neoplasms

• Assess cellularity & slide quality
• Determine there are no (few) neutrophils
• Determine if cells are individual or in clusters
• Cell shape
• Nuclear shape
• Features of malignancy

Epithelial

Tight clusters
Highly cellular

Polygonal
Central nucleus
Mesenchymal
Spindle-shaped
Eccentric nucleus
Individual to aggregated
Low cellularity

Round Cells
Round
Variable nuclear location
Individual cells
Highly cellular

Guess the cell type?
Epithelial

Photo courtesy of Dr. Mike Fry, UTCVM
Cytologic Features of Malignancy

- Hypercellularity (decreased cohesiveness)
- Pleomorphism (anisocytosis, anisokaryosis)
- High/variable N:C ratio
- Multinucleation
- Karyomegaly
- Mitoses (+/- bizarre)
- Nuclear molding (rapid cell growth)
- Coarse nuclear chromatin pattern
- Large, angular, or variable nucleoli (anisonucleolosis)

Key word: variability
Cytologic Features of Malignancy

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- Nuclear molding (rapid cell growth)
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- Large, angular, or variable nucleoli (anisonucleolosis)

Nuclear features more reliable than others for diagnosis of malignancy

Criteria of Malignancy

Anisokaryosis

Anisocytosis

High N:C ratio

Benign Prostatic Hyperplasia

Prostatic Carcinoma

Benign Prostatic Hyperplasia

Prostatic Carcinoma
Variable N:C

Anisonucleolosis

Criteria of Malignancy

Prominent, multiple, & variably-sized nucleoli

Nuclear molding

Multinucleation
Nuclear blebs

Normal small lymphocyte
Neoplastic lymphocyte
Altered chromatin pattern

Aberrant mitotic figures
Mitotic Figures – can be normal!!

Metaphase

Anaphase

Mitotic Figures

Atypical vacuolation or inclusions

Pink inclusions

TCC & prostatic carcinoma can have these inclusions

Loss of normal components

Malignant melanoma – note lack of melanin
Benign Epithelial Tumors

Mammary adenoma in a bitch
Uniform nuclear size & consistent N:C ratio

Perianal (hepatoid) gland adenoma in a dog

Both of these are “perfect” examples of cell-to-cell adhesion: sheets and uniformity of cells and nuclei with distinct cell borders!

Benign Epithelial Tumors

Epithelial

Hepatocytes
mild glycogen accumulation

Benign

Malignant Epithelial Tumors
Mesenchymal malignant

Malignant Mesenchymal Tumor

Fine needle aspirate from the lung of a dog with histiocytic sarcoma

Note the large binucleate cell with massive nuclei and multiple, irregular shaped nucleoli.

Note phagocytized erythrocytes (a feature of malignant histiocytic tumors)

The erythrocytes & neutrophils in the field provide an indication of the size of the tumor cell.

A word of caution ......

Reactive, benign, fibroblasts can have markedly atypical morphology & easily get mistaken for malignancy.
**Diagnostic Tree**

- Mass
  - Inflammatory
    - Neutrophils
    - Eosinophils
    - Mixed
  - Healthy
  - Degenerate
  - Non-inflammatory
    - Non-neoplastic
      - Neoplasia
    - Cyst
    - Hematoma
    - Sialocele
    - Mesenchymal

**Round Cell Tumors**

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Monomorphic Lymph population</th>
<th>Mast Cell Tumor</th>
<th>Metachromatic granules</th>
<th>Eosinophils; Diff-Quik</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast Cell Tumor</td>
<td>Metachromatic granules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histiocytoma</td>
<td>Plain; ‘fried egg’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>Eccentric nucleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVT</td>
<td>Ropey chromatin; vacuoles</td>
<td></td>
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</tr>
</tbody>
</table>

**11-year-old Labrador Retriever**

Granules do not always stain with Diff Quik.

Some tumors just don’t have cells with many granules.
Mast cell tumors

Canine mast cell tumors:
- mast cells
- eosinophils
- reactive fibroblasts

Variable granule staining

Neoplasms With Cytoplasmic Granules
- Mast cell tumor
- Melanoma
  - Black, green, blackish-green granules
  - Variable numbers of granules
  - Pleomorphic cells
    - Round
    - Polygonal
    - Spindle
    - Mixture of shapes
Melanoma
Often difficult to classify as round, epithelial or spindle

“Amelanotic”
Fontana-Masson
Bring what you know to the cytology specimen

Histiocytoma
Small lymphs
Pale cytoplasm
Boring?
Plasma cell tumor

Blue cytoplasm
Perinuclear clearing
Eccentric round nucleus
Bi or multinucleation
Moderate anisokaryosis

Flame cells

Plasma cells resemble osteoblasts!!

Malignant PCT

Plasma cell tumor
TVT punctate vacuoles

look lymphoid to histiocytic

ropey, coarse chromatin

Melanoma
Large 'inguinal' mass in 12 year old, grey, horse.

Lymphoma

Lymphoglandular bodies

Lymph Node Cytology

• Indications:
  – Lymphadenomegaly
  – Suspected metastasis
• Normal size = normal node = do not aspirate!

Lymph Node Cytology Technique

• Popliteal and prescapular are preferred sites to sample in generalized lymphadenopathy
• Avoid the center of very large lymph nodes (aim tangentially)
• FNB preferred over FNAB (less blood contamination)
• Be very gentle with smears – immature lymphoid cells are fragile
• Avoid formalin like the plague 😊
Normal lymph nodes (don’t aspirate):

**Small lymphocytes** are 75-90% of nucleated cells
- Slightly larger than canine erythrocyte & smaller than neutrophil
- Dense chromatin
- Can look like small cell lymphoma

**Large lymphocytes:**
- < 20% - (2 in this photo)
- Larger than neutrophil
- Often up to 4x size of a RBC
- Chromatin less dark
- Nucleoli often visible
- More cytoplasm (blue)

**Lymphoglandular Bodies**
- Small blue droplets in the background that are fragments of cytoplasm and common in lymphoid hyperplasia and neoplasia.

**Lymphadenomegaly**
- Hyperplasia (reactive) – antigenic stimulation
- Inflammation – lymphadenitis
- Primary neoplasia – lymphoma
  - Monomorphic population
  - Typically medium to large lymphocytes >50% (but can have small cell lymphoma)
  - Few to no plasma cells or other cells
- Metastatic neoplasia
Reactive Lymph Node (Hyperplasia)

- Predominantly small lymphocytes
- Increase in plasma cells (5-20%)
- Medium and large lymphocytes increased, but still <20%

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Small Lymphocytes</th>
<th>Lymphoblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC diameter</td>
<td>1.0-1.5 X</td>
<td>2-3 X</td>
</tr>
<tr>
<td>Neutrophil diameter</td>
<td>Smaller</td>
<td>Larger</td>
</tr>
</tbody>
</table>

Lymph Node Aspirate---Interpretation?

Plasma cells (small arrows), large lymphocyte (large arrow), small lymphocytes (small arrowhead), and Mott cell (large arrowhead).

Reactive Lymph Node
Lymphoma

- Monomorphic population of medium to large lymphocytes (>50% of cells); decreased small lymphocytes; rare plasma cells
- May have numerous lymphoglandular bodies (cytoplasmic remnants)
- Presence of mitotic figures variable

Lymph Node Metastasis

- Metastasis of any malignant tumor is a possibility
- Epithelial cells – Easy to confuse with macrophages or accidental aspiration of submandibular salivary gland
- Cytology is as accurate as histopathology in predicting presence or absence of metastasis
Lymph Node Aspirate----Interpretation?

Metastatic Carcinoma

Clusters of large malignant epithelial cells (large arrow) resembling macrophages, small lymphocytes (large arrowheads), erythrocytes and vacuolated macrophage (small arrowhead).

Aspirate of submandibular lymph node with metastatic fibrosarcoma. Note spindle shaped fibroblasts (arrows) and small lymphocyte (arrowhead).
Metastatic mast cell tumor in lymph node: Mast cells are indicated by arrows and small lymphocytes by arrowheads.

Mast Cell Tumor Metastasis to Lymph Node

- Rare individual mast cells: Reactive hyperplasia (e.g. draining allergic inflammation)
- 2 to 3 aggregates of 2 or more mast cells: Possible metastasis
- 4 or more aggregates of 2 mast cells; or 2 or more aggregates of more than 4 mast cells: Probable metastasis
- 6 or more aggregates of more than 3 mast cells; or 5% mast cells in specimen: Almost certain metastasis
- Beware of false negatives

DIF-Quik - poorly staining metastatic mast cells vs. traditional Wright's stain
Lymphadenitis
- Suppurative: Increased neutrophils, which may be degenerate if bacteria are in the node
- Eosinophilic
- Granulomatous: Increased macrophages
- Specific etiologic agents may/may not be present

Granulomatous lymphadenitis: Macrophages (arrows) & neutrophils (arrowheads) with few lymphocytes

Lymph node aspirate from dog: Almost all cells are macrophages (large arrows) filled with clear, non-staining organisms (small arrows).
A few small lymphocytes are present (arrowhead).
Same lymph node as previous stained w/ acid-fast stain.

*Mycobacteria* are pink & intracellular (macrophages-large arrows).

Few small lymphocytes (arrowhead) & neutrophils (small arrow).

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**Summary: Round Cell Tumors**

- Mast cell tumor – granules; eosinophils
- Histiocytoma – young dog, abundant pale blue cytoplasm; kidney-shaped nuclei
- TVT – location; looks like histiocytoma
- Lymphoma – scant cytoplasm; blast cells
- Plasma cell – eccentric nuclei, Golgi zone, abundant cytoplasm

- **If in doubt, excisional biopsy**
  - at least you ruled out inflammation and ruled in round cell tumor

---

**Diagnostic Tree**

- **Mass**
  - **Inflammatory**
    - Neutrophils
      - Healthy
      - Degenerate
  - Eosinophils
  - Mixed
- **Non-inflammatory**
  - Non-neoplastic
    - Neoplasia
      - Hyperplasia
    - Cyst
    - Hematoma
    - Stalolele
  - Round
  - Epithelial
  - Mesenchymal
Epithelial Cell Tumors

- Adenoma vs. carcinoma
- Sebaceous gland tumors
- Mammary neoplasms
- Prostatic neoplasms
- Nasal tumors
- Transitional (urothelial) cell tumors
- Perianal neoplasms
- Basaloid epithelial tumors

Epithelial Tumors

- Cells in sheets or clusters
- Usually many cells present
- Cytoplasmic borders usually distinct
- Often large cells w/ abundant cytoplasm
- May show signs of differentiation

Adenoma: Uniform cells. Acinar formation on far right.

Mass on the Prepuce of a Dog

Benign or malignant?

Squamous cell carcinoma

Note neutrophils
Diagnosis?

Perianal/hepatoid adenoma adenoma
(not anal sac adenocarcinoma)

- Hormonally dependent
- May regress after castration; castration may prevent recurrence

Adenocarcinoma of the Apocrine Gland of the Anal Sac

- Female dogs
- Hypercalcemia
- This tumor was small but had already advanced into the pelvic inlet and metastasized
Adenocarcinoma of the Apocrine Gland of the Anal Sac

- Cells from this tumor cells often look bland and benign
- Knowing the biologic behavior of certain tumors is a better predictor of malignancy than looking at cytology or histology in this case
- These tumors tend to produce naked nuclei

Adenocarcinoma of the Apocrine Gland of the Anal Sac

Abdominal Metastasis

Inflammatory or Neoplastic?
Shape of cells and nuclei?
Variability?

Dog, urine
Transitional Cell Carcinoma

Diagnostic Tree

Mesenchymal (Spindle) Cell Tumors

- 'oma vs. sarcoma
- Fibroma / Fibrosarcoma
- Osteoma / Osteosarcoma
- Hemangioma / Hemangiosarcoma
- Peripheral nerve sheath tumor (hemangiopericytoma) vs. perivascular wall tumor difficult to distinguish histologically
  - PWT: veiled cells, crown cells
Mesenchymal Tumors

- Exfoliate poorly in FNAs and imprints; few cells present
  - Exceptions: PNST, osteosarcoma, feline vaccine site sarcomas
- Usually elongated nuclei
- Cytoplasmic tails (spindle cells)
- Usually individual cells but sometimes clusters with intercellular matrix
- Active fibroblasts resemble malignant mesenchymal cells

Mesenchymal Tumor

Fine needle aspirate from a firm mass in the skin of a dog.
Fusiform cells, suggesting either mesenchymal tumor or granulation tissue
Diagnosis: Peripheral nerve sheath tumor (hemangiopericytoma) made with histopathology evaluation.
Mesenchymal lesions should be submitted for histopathology as active fibroblasts are difficult to differentiate from neoplastic fibroblasts and may even have features that suggest malignancy.

Ten cm diameter, non-ulcerated mass; hind leg of dog

History of a mass is key
Mesenchymal tumor is more likely here than granulation tissue
Cell variability indicates malignancy
Recommend histopathology (incisional vs. excisional biopsy)
Cytology vs. Histopathology: Diagnosis?

Mesenchymal Tumors

- Don’t worry about the exact name
- Grading schemes DO NOT differentiate based on the name:
  - fibrosarcoma, hemangiopericytoma, neurofibrosarcoma, peripheral nerve sheath tumor, perivascular wall tumor, poorly differentiated sarcoma etc.
- Grading schemes are based on histopathology: degree of differentiation, necrosis, and mitotic index (some use other criteria)

**Prognostic Factors for Cutaneous and Subcutaneous Soft Tissue Sarcomas in Dogs**

*Vet Pathol.* 2011;48(1):73-84

<table>
<thead>
<tr>
<th>Type</th>
<th>Tissue of Origin (Histogenesis)</th>
<th>Phenotype</th>
<th>Histologic Habitus</th>
<th>Typical Immunohistochemistry (%)</th>
</tr>
</thead>
</table>
| Fibrosarcoma                  | Fibrous tissue                  | Fibroblast, fibrocyte | Treatment bundles, fascicles, some patterns | Vimentin+ (90%)
| Hemangiopericytoma            | Perivascular wall               | Lipoblasto, lipocyte | Treatment bundles, fascicles, some patterns | Vimentin+ (90%)
| Neurofibrosarcoma             | Perineural, perineural          | Neurone, Schwann cell, neurofibroblast | Treatment bundles, fascicles, some patterns | Vimentin+ (90%)
| Peripheral nerve sheath       | Schwann cell                    | Neurofibroblast | Treatment bundles, fascicles, some patterns | Vimentin+ (90%)
| Perivascular wall tumor       | Periarterial, perineural         | Vascular smooth muscle, including myepihyseal cells, perivascular occluding | Treatment bundles, fascicles, some patterns | Vimentin+ (90%)
| Mesenchymal sarcoma (malig-  | Any mesenchymal tissue          | Monophasic or biphasic or biphasic | Treatment bundles, fascicles, some patterns | Vimentin+ (90%)

**Sarcoma:**

- Prognostic factors include:
  - Histologic type
  - Degree of differentiation
  - Necrosis
  - Mitotic index
  - Size

**Prognostic Factors for Cutaneous and Subcutaneous Soft Tissue Sarcomas in Dogs**

*Vet Pathol.* 2011;48(1):73-84
Canine Soft Tissue Sarcomas

Mitotic index, independent of grade, provides important prognostic information (>9 per 10 hpf = reduced survival)

Grade is more informative regarding likelihood of recurrence
- No clear consensus on STS grade vs. survival
- Complete margins predict nonrecurrence
- Wide or radical excision → lowest recurrence rate, but relationship to survival not yet proven so not sure if necessary
Radiograph of a Cat

Intramedullary pin
Mass
Bone lysis – femur is gone!
Differential diagnosis?
Osteomyelitis
Osteosarcoma
Other neoplasm
Plan: Aspirate for cytology and culture

Notice nucleus almost hanging out of cell
This is not osteomyelitis, as there are no neutrophils
Culture not needed
Osteosarcoma most likely
Matrix (osteoid)
Lytic Bone Lesion From a Dog

Neutrophils? Are cells individualized or organized? Shape of cells? Shape of nuclei? Variability? Any other diagnostic clues?

Diagnosis: Osteosarcoma

Keys to diagnosis:
1. LOCATION: bone with lysis
2. Morphology: spindle cells
3. Product: note the pink material in both specimens; this is osteoid (very useful diagnostically)

Osteosarcoma vs. Multiple Myeloma

OSTEOSARCOMA
• Lame, thin, sick
• One bone, one lesion
• Giant breeds (dog)
• Characteristic locations
• Radiographic pattern: Osteolysis and osteoproduction
• Normocalcemia & protein
• Osteoid; variability; giant cells

MULTIPLE MYELOMA
• Lame, thin, sick
• Multiple bones; multiple lesions per bone
• Radiographs: Multiple foci of osteolysis; discrete lytic areas
• 10% are hypercalcemic
• Monodonal gammopathy
• Round cells with a distinct Golgi zone
6-year-old cat with mass at the base of the neck. Fine needle aspirate

Diagnosis: Injection site sarcoma; fibrosarcoma with giant cells; giant cell tumor of soft parts

Keys to diagnosis: cat, anatomic location, cell appearance

Questions so far??

http://imgur.com/gallery/4i6o9o7

Questions so far??
A formal diagnostic write up includes:

1. **Description**

2. **Interpretation**
   - bacterial infection with marked neutrophilic inflammation

3. **Comment**
   - consider culture & sensitivity to help direct antibiotic therapy

**Cytology Description**

- Cytology description should include:
  - Cellularity
  - Cell distribution
  - Predominant cell type with description (shape, arrangement, nuclei, cytoplasm, cell borders, features of malignancy)
  - Organism descriptors
  - Other cell types, relative numbers
  - Background (blood, color, lymphoglandular bodies, free granules, etc)
  - Contaminants
Example

• This fair quality, moderately cellular cytologic specimen is composed of numerous monomorphic round cells with distinct cell borders, abundant pink cytoplasm which is filled with small purple granules, and round, centrally placed purple nuclei with finely stippled chromatin and distinct nucleoli. Rare mitotic figures are observed. These are admixed with moderate numbers of eosinophils and rare spindle cells on a background of pale blue proteinaceous fluid.

• Impression: Mast cell tumor.

Bone marrow description

• Every good bone marrow description should include the following:
  – Quality
  – Cellularity
  – Hematopoietic to adipose ratio
  – M:E ratio
  – Proliferating pool vs. maturation/storage pool
  – Megakaryocyte numbers
  – Iron storage pool
  – Organisms
  – IMPRESSION!

Example

• This excellent quality, densely cellular bone marrow specimen is composed of roughly equal proportions of adipose tissue and hematopoietic tissue. M:E ratio is approximately 1.5:1. Roughly 80% of the myeloid cells are metamyelocytes, bands and segmenters (maturation pool), and 20% are within the proliferative pool. Roughly 90% of the erythroid cells are rubricytes and metarubricytes (maturational pool), and the remaining 10% are within the proliferative pool. There are adequate numbers of immature and mature megakaryocytes, and numerous hemosiderin laden macrophages (iron storage pool).

• Impression: Normal bone marrow.
Blood smear description

- Every good blood smear description should include the following:
  - Quality
  - Cellularity
  - WBC differential
  - Platelet evaluation
  - RBC evaluation
  - Organisms
  - IMPRESSION!

Outline

- Introduction
- Techniques
- Interpretation
- Description
- Case examples

- Adult cat with swelling over bridge of the nose
- Fine needle aspirate
- What's the first question?
Cause: *Cryptococcus neoformans*
Six-year-old dog

- Labored breathing
- Thoracic radiographs: Interstitial pneumonia
- Transtracheal wash

Pyogranulomatous Inflammation
Blastomycosis
Cutaneous mass from a dog
Plasma cells predominate
- Mild anisocytosis
- Moderate anisokaryosis
- Intermediate N:C
- Deep blue cytoplasm often with a pink rim
- Small perinuclear clearing
- Eccentric, round to ovoid nucleus
- Dense chromatin
- Nucleoli inconspicuous
- Rare binucleated cells

Highly cellular
Cells present in large, dense aggregates and individually.
Faint pink, hemodiluted, stippled pink, background.

Interpretation: Plasma cell tumor

Subcutaneous mass, ventral cervical region dog
• Highly cellular
• Faint purple, densely hemodiluted background w/a few free nuclei, small lymphs & vacuolated macrophages containing blue-black material
• Cells present individually and in dense clusters
• Epithelial cells predominate with round to indistinct cell borders
• Cells occasionally surround pink colloid like material
• Rare acini-like structures present; contain colloid
• Mild anisocytosis and anisokaryosis
• Cells have pale blue cytoplasm & a few blue-black granules (tyrosine granules)
• Central nuclei, dense chromatin, inconspicuous nucleoli

Interpretation: Thyroid neoplasm, follicular carcinoma most likely
Enlarged right mandibular lymph node, dog
• Very highly cellular
• Dense confluence of cells
• Light blue background containing small amounts of blood and scattered free mast cell granules, rare lymphoglandular bodies
• Mast cells predominate
• Exhibit moderate anisocytosis and anisokaryosis
• Pale blue cytoplasm, poorly to densely granulated, rarely vacuolated.
• Often eccentric round to ovoid nuclei, smooth chromatin nucleoli not appreciated
• Few mitotic figures
• Many eosinophils, rare neutrophils, very rare plasma cell

Interpretation: metastatic mast cell tumor

• 10-month-old shepherd mix
• Hairless, round, 1.5cm raised cutaneous mass on pinna
• Submitted FNAs
<table>
<thead>
<tr>
<th>Type</th>
<th>Cellularity</th>
<th>Arrangement</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round cell</td>
<td>High</td>
<td>Individual</td>
<td>Round</td>
</tr>
<tr>
<td>Epithelial</td>
<td>High</td>
<td>Clusters</td>
<td>Polygonal*</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>Low**</td>
<td>Individual</td>
<td>Spindle</td>
</tr>
</tbody>
</table>
• Canine cutaneous histiocytoma
• Benign dermal neoplasms
• Langerhans cell origin (histiocyte)
• Typically grow rapidly and spontaneously regress
- Moderately cellular
- Cells present individually & in dense aggregates
- Capillary fragment admixed with aggregate
- Spindloid cells predominate
- Low N:C, wispy, veiling, often indistinct cell borders
- Mild to moderate anisocytosis & anisokaryosis
- Pale blue cytoplasm, very rare vacuolation
- Rare intracytoplasmic pink globule (or nuclear bleb)
- Eccentric round to ovoid nucleus
- Stippled chromatin
- Inconspicuous nucleolus
- Rare binucleated cells
- RBCs & cellular debris in background

**Soft tissue sarcoma: morphology consistent with perivascular wall tumor (soft tissue sarcoma)**

**Cutaneous mass, dog**
Highly cellular
- cells present individually & dense aggregates
- round to spindloid cells predominate
- low N:C
- marked anisocytosis and anisokaryosis
- abundant blue-grey cytoplasm
- occasionally contains abundant melanin granules
- variably placed round to ovoid nuclei
- very coarse chromatin
- multiple, prominent and pleomorphic nucleoli
- bi and multinucleated cells present
- light blue vacuolated background containing rare RBC, rare rod-shaped bacteria, low numbers of neutrophils, rare keratin scroll.

Malignant melanoma
Mass under tongue, cat
- Low to moderate cellularity
- Cells present individually & dense disorganized clusters
- Pleomorphic, round to angular to elongate epithelial cells
- Low N:C
- Marked anisocytosis and anisokaryosis
- Faint blue to dark blue/sky blue keratinized cytoplasm; often vacuolated with vacuoles occasionally concentrated around the nucleus
- Exhibit occasional dyskeratosis (mismatch between nuclear and cytoplasmic maturation)
- Large, round, central nucleus with coarse chromatin and rarely a large, prominent nucleolus
- Light blue, hemodiluted background containing small amounts of free keratin and streaming nuclear debris
- Mild neutrophilic inflammation & neutrophilic emperipolesis

**Squamous cell carcinoma**

**Abdominal fluid from a horse**

**Interpretation?**
Abdominal fluid from a horse

Numerous degenerate neutrophils & macrophages with intracellular bacilli

Note toxic changes in PMNs

Septic exudate with bacteria

Splenic Aspirate from a Cat

• Merozoites in severely enlarged macrophages
• Piroplasms in erythrocytes

Cytauxzoonosis
Abdominal aspirate from dog with ascites

- Few small clusters & some individualized epithelial cells
- Cells are molded or occasionally wrap each other
- Large variation in cellular and nuclear size and shape
- Very prominent, large nucleoli.

Malignant neoplasm, consistent with carcinoma

- Dog with lytic bone lesions, monoclonal gammopathy and hypercalcemia
Interpretation?

Multiple myeloma

Questions?