Descriptive Cytology

Shannon H. Lacy, DVM, MPH, DACVP, DACVPM
JPC, Veterinary Pathology Service
Silver Spring, Maryland

Descriptive Veterinary Pathology
C.L. Davis DVM Foundation
Auburn, Alabama
June 2013

Agenda

• Overview:
  – Advantages
  – Limitations
• Techniques
• Interpretation
• Description
• Case examples
Overview

Cytology

Histology

Impression smear

Cytology

Advantages

• Fast
• Cheap
• Relatively non-invasive
  – Anesthesia not needed for cutaneous masses
• Basic interpretation can be performed in clinic
  – Establish the diagnosis (or differential diagnosis)
  – Inform the treatment plan
• Better than histopathology for some lesions

Limitations

• Samples are not always representative
• Cannot assess architecture of the lesion
• May not be able to exclude malignancy
• Cytologist’s skill and experience
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-inflamatory / 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)
Implications

- **Board exam preparation** should focus on first two lists on preceding slides
- Need to communicate the limitations of cytology to clients/contributors

Agenda

- Overview:
  - Advantages
  - Limitations
- **Techniques**
- Interpretation
- Description
- Case examples

Techniques

- **Specimen management** affects accuracy of observation
  - Representative specimen
  - Proper application to glass slide
  - Sample handling (**no formalin!**)
  - Adequate staining
  - Examination with a clean, functioning microscope
Techniques

• Fine-needle biopsy (FNB) / fine-needle aspiration biopsy (FNAB)
• Touch imprint
• Scraping

Regardless of sampling technique, dry-wipe the surface of the glass slides to remove “invisible” glass particles that interfere with spreading.

FNAB / FNB

• Most common, easiest to obtain
• 20- or 22-gauge needle, 1 or 1.5 inch
• 2.5-3.5 inch spinal needle for deeper organs, mouth; leave stylet in place to protect from contamination
• +/- attached to a 6 or 12-cc syringe
   – Retract plunger slightly (1/2 to 1 cc vacuum)
• Practice makes perfect!
FNAB / FNB → Squash Prep

Touch Imprint

- 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

Touch Imprint

- Aggressively debride ulcerated surfaces first
- Otherwise, true etiology obscured by contaminants (debris, bacteria, inflammation)
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
- Handle fluid with a plasma-like consistency as you would a blood smear
- When possible, make direct, centrifuged (buffy coat) and cytospin preps
- Refractometer-determined TS (protein) needed for all pleural & peritoneal fluids to correctly classify the fluid

Helpful Hints

- Good preparation is the key to success
- Avoid formalin fumes
- Impression smear: touch, don’t wipe!
- Prepare thin smears (monolayer)
- Don’t apply pressure when preparing smears
- Put liquid samples into EDTA-tubes ASAP
  - No fluid samples to JPC, but we welcome the cytology from them
- Air-dry slides well before shipping or staining
- Always include anamnesis
Staining

• Let dry completely first
• Follow stain instructions

Diff Quick (Modified Wright Giemsa)

• Great cytoplasmic detail
• Progressive staining ability
• Mast cell granules may not stain well
• Frequent use → contamination

Wright Stain

• Blood smears
• Complex technique
New Methylene Blue

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

Giemsma

- Mast cell granules (esp. in cats)
- Infectious organisms
  - Bacteria
  - Fungi
  - Myxosporea
  - Apicomplexa
Papanicolaou

- Fluid cytology
- Vaginal swabs
  - Nuclei: black
  - Keratin containing cells: yellow
  - Superficial epithelium: pink
  - Parabasal cells: green/blue
  - Metaplastic cells: green and pink
- Complex technique

Other Stains

- Gram stains
- Acid fast stains
- Immunocytochemistry
- Can be done as counterstains

Coverslip!

- Needed to focus at 40x objective
- Can use a drop of oil on top of coverslip if you need to go to 100x
- “Short cut” = dry coverslip on top of slide
Coverslip!

No Coverslip / Coverslip

Coverslip AND Oil!
Common Technical Problems

- Diagnostic material wiped off while trying to remove oil!
- Inadequate drying before fixation
- Poor cell preservation
- Rolled keratinocytes
- Formalin exposure
- Hypocellularity
- Too thick

Agenda

- Overview:
  - Advantages
  - Limitations
- Techniques
- Interpretation
- Description
- Case examples

Interpretation

- Meet your new friends...
Interpretation

• Scan entire slide at low magnification
• Ensure adequate staining
• Find areas of cellularity to examine on high magnification (40x)
  – Oil rarely needed!
• Always begin by assessing specimen quality and cellularity

Slide Quality & Cellular Preservation

• Formalin exposure
  – Avoid exposure to fumes during preparation
  – Ship in separate container
• Wet prep slides
  – Ensure adequate drying time
  – Use hair dryer if in a hurry
• Inadequately fixed specimens
  – Fix before shipping
  – Slides with lots of lipid/wax won’t stain well
Freeze Artifact

Staining

Not enough  Just right  Too much
Most spindle cell tumor cytologies are paucicellular.
Bacteria / Yeast

Name & Significance?

Diagnostic Tree

Mass

Inflammatory

Neutrophils

Eosinophils

Mixed

Non-inflammatory

Cyst

Hematoma

Sialocele

Round

Epithelial

Mesenchymal

Non-neoplastic

Neoplasia

Hyperplasia

Healthy

Degenerate
Inflammation

- Various species
  - Purulent inf.
  - Abscess

- Bacteria?
  - >90% neuts?
  - >80% macs?

- Fungi?
  - Chronic infection
  - Immune mediated
  - Foreign body
  - Resolving infection

- Mycobacterium
- Nocardia
- Actinomycetes

- Histoplasma
- Blastomycetes
- Sporothrix
- Pythium
- Aspergillus

Inflammatory Lesions

- Next question is: “Do neutrophils predominate?”
  - If yes, consider:
    - Abscess
    - Bacterial infection
    - Foreign body
  - Is etiology evident?

Cytologic Evaluation of a “Lump”

- First question to ask, “Are there neutrophils?”
  - If yes, then lesion is inflammatory
  - If no, then lesion is a cyst or hyperplasia/neoplasm

- Fungi?
  - Chronic infection
  - Immune mediated
  - Foreign body
  - Resolving infection

- Histoplasma
- Blastomycetes
- Sporothrix
- Pythium
- Aspergillus

- Chronic infection
- Immune mediated
- Foreign body
- Resolving infection

- Histoplasma
- Blastomycetes
- Sporothrix
- Pythium
- Aspergillus
Microbial Etiologies

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

Neutrophil (15 μm) & Up
- Cryptococcus
- Blastomyces
- Rhinosporidium
- Coccidioides
- Hyphae
  - Aspergillus
  - Mucor
- Parasites

Case Study

- Adult cat
- Swelling over bridge of the nose
- Fine needle aspirate
- What’s the first question?

Inflammatory or neoplastic?

Inflammatory cell types?
Neutrophils and macrophages primarily
• Cause?

Macrophages contain oval organisms with a non-staining capsule

Cryptococcus neoformans

Cryptococcus neoformans

Six-year-old dog
• Ulcer on foot
• Labored breathing
• Thoracic radiographs: Interstitial pneumonia
• Aspiration of foot lesion
• Transtracheal wash
Are there neutrophils?

Organisms = blue extracellular blobs larger than neutrophils.

Blastomycesis

Multinucleated giant cell suggests fungal infection. Intracellular blue yeasts.

Blastomycesis

Pyogranulomatous Inflammation
Blastomycesis
**Histoplasmosis**

- Numerous small oval organisms
- Intracellular (intrahistiocytic)
- Eccentric nucleus surrounded by a halo

**Histoplasma capsulatum**

**Fungal Hyphae**

Often don't stain and appear as ghosts or tracks
**Fungal Hyphae**
- Nasal mass or osteolysis
- Bone
- Lung
- Stomach
- Eye, especially in horses

**Fungal Keratitis--GMS**

**Leishmania sp.**
- Often present in large numbers
- Diagnostic key: small rod-shaped kinetoplast near the nucleus in each organism
- *Histoplasma capsulatum* lacks a kinetoplast
Toxoplasma sp.

Toxoplasma gondii organisms in macrophages (above) and free tachyzoites (right) in a tracheobronchial wash from a cat.

Elongated; banana shaped
Low numbers
Hard to find!

Malassezia pachydermatis

- Small budding yeasts shaped like “footprints” or “peanuts”
- Small numbers in ear swabs from clinically normal dogs and cats
- When in large numbers (>10 per HPF), they are likely to be significant
- Also involved in some exudative skin conditions of dogs, as a primary or a secondary etiologic agent

Equine Transtracheal Wash

- Eosinophils
- Mast cells
- Curschmann's spirals
- Foamy macrophages
- Lymphocytes

Diagnosis?
**Eosinophils**

- If >20% of nucleated cells, suspect:
  - Parasite
  - Immune-mediated/allergic
  - Neoplasia (mast cell tumor, lymphoma)
  - Collagenolytic disorders (cats and horses)
- Eosinophils on endometrial cytology in horses is associated with pneumovagina and pneumouterus
- Eosinophils do not always have brilliant red granules, especially if stained with new methylene blue

**Feline Eosinophils**

...have rust-brown granules

**Diagnostic Tree**

Mass

- Inflammatory
  - Neutrophils
    - Healthy
    - Degenerate
  - Eosinophils
  - Mixed
- Non-inflammatory
  - Neoplastic
    - Cyst
    - Hematoma
    - Sialocele
  - Neoplasia
    - Hyperplasia
    - Round
    - Epithelial
    - Mesenchymal
If there are few to no neutrophils:
- Cyst
- Seroma/Hygroma
- Hematoma
- Sialocele
- Neoplasm

“Keratinizing Cyst or Neoplasm”
- Follicular cysts:
  - Epidermal inclusion cyst
  - Infundibular cyst
  - Isthmus cyst
  - Panfollicular cyst
- Follicular neoplasms:
  - Infundibular keratinizing acanthoma
  - Trichoepithelioma
  - Pilomatricoma

“Keratinizing Cyst or Neoplasm”
- Common in dogs
- Lined by stratified squamous epithelium
- Filled with squames and keratin debris
- Rectangular cholesterol crystals are common
  - Crystalization of cholesterol from dead cells
  - May look like a vaginal preparation because of the keratinized squames and amorphous cellular debris
  - Neutrophils
  - Cholesterol crystals
Hematoma vs. Hemorrhage

• Platelets = blood contamination
• Erythrophagocytosis, erythrocyte aging, hemosiderin and xanthochromia = hematoma

Erythrophagocytosis + hemosiderin pigment
Platelets indicate blood contamination, rather than hematoma (or hemoabdomen/hemotorax, etc).

Hematoma vs. Hemorrhage

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

Windrows
Cholestasis

Diagnostic Tree

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
Neoplasms

<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>

* Some epithelial cells spindle (e.g. trichoblastoma)
** A few mesenchymal neoplasms are exfoliative (e.g. PNST)

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)

Kew word: variability
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)

Nuclear features more reliable than others for diagnosis of malignancy

Cytologic Features of Malignancy

At least 3 nuclear criteria of malignancy should be present for a confident diagnosis of malignancy....

Unless you know the biologic behavior of the tumor in question
e.g. if it is an oral melanoma you don’t need any of the above

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!
Malignant Epithelial Tumors

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.

Malignant Mesenchymal Tumor

Diagnostic Tree
Round Cell Tumors

- Lymphoma
- Mast cell tumor
- Plasmacytoma
- Histiocytoma
- Transmissible venereal tumor
- Melanoma

Cells are usually individual
- Sometimes close together and appear to be in small aggregates (mimic epithelial tumors)
- Usually many cells are present
- Round cells with round nuclei
- Distinct cytoplasmic borders
- Cells may be well differentiated (mast cell tumors)

11-year-old Labrador Retriever

Fine needle aspirate from a mass on the lip
Numerous large round cells with round nuclei
Diagnosis?
Variable numbers of metachromatic (purple) granules typical of neoplastic mast cells
Mast cell granules do not always stain with Diff Quik
Variability of Mast Cell Staining

Urine from a male dog

Giemsa – can be technically difficult
Toluidine Blue
Immunocytochemistry

Neoplasms With Cytoplasmic Granules

- Mast cell tumor
- Melanoma
Neoplasms With Cytoplasmic Granules

- Mast cell tumor
  - Round cell
  - Purple granules
  - Variable numbers of granules
    - From many to none

- 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

FNA, left ear of a 2-year-old female dog

• Inflammatory or neoplastic?
• Predominant nucleated cell type?

Transmissible venereal tumor

Fine needle aspirate from a mass on the penis of a dog

Uniform population of round cells with variable nuclear size, coarse chromatin pattern and discrete cytoplasmic vacuoles.

The large cell is a non-keratinized squame from the penile or preputial mucosa.

Diagnosis?
**TVT:** round cells, more cytoplasm than in lymphoma, look like histiocytoma

*Location is KEY!*

---

**Plasma Cell Tumor**

*Skin, Oral, GI*

If in bone = multiple myeloma

Round cell tumor, eccentric nuclei, abundant blue cytoplasm, pale Golgi zone, variable nuclear size, multinucleate cells

*Diagnosis?*

Plasma cells resemble osteoblasts!

---

**PCT:** Most are behaviorally benign; aggressive tumors infrequently occur
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 😊
Lymphadenomegaly

- Hyperplasia (reactive) – antigenic stimulation
- Inflammation – lymphadenitis
- Primary neoplasia – lymphoma
- 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>

Other

- Lymphoma
  - Lymphoblasts >50%
  - Few to no plasma cells or other cells
- Normal node (don’t aspirate!)
  - 75-90% small lymphocytes, dense chromatin
    - Can look like small cell lymphoma
- Lymphadenitis (inflammatory cells +/- cause)
- Metastatic neoplasia
Lymph Node Aspirate—Interpretation?

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

Reactive Lymph Node

FNA from an Enlarged LN—Interpretation?
Lymphoma

- Increased lymphoblasts (>50% of cells); decreased small lymphocytes; rare plasma cells
- May have numerous lymphoglandular bodies (cytoplasmic remnants)
- Presence of mitotic figures variable

Lymphoglandular Bodies

Small blue droplets in the background that are fragments of cytoplasm and common in lymphoid hyperplasia and neoplasia.
Fine Needle Aspirate of a Lymph Node—Interpretation?

Normal lymph nodes:
DON’T aspirate

- Small lymphocytes are 75-90% of nucleated cells
- Slightly larger than canine erythrocyte & smaller than neutrophil
- Dense chromatin

Lymphoblasts:
- < 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)
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----interpretation?
Aspirate of submandibular lymph node with metastatic fibrosarcoma. Note spindle shaped fibroblasts (arrows) and small lymphocyte (arrowhead).

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

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
- 5 or more aggregates of more than 3 mast cells; or 5% mast cells in specimen: Almost certain metastasis
- Beware of false negatives

Metastatic mast cell tumor stained with Diff-Quik: Poorly staining mast cells indicated by large arrows; plasma cell by large arrowhead; small lymphocytes by small arrow.

Aspirate of same node but now stained w/ traditional Wright's stain.
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).

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
    - Neutrophils
    - Eosinophils
    - Mixed
      - Healthy
      - Degenerate
    - Non-neoplastic
      - Cyst
      - Round
      - Hematoma
      - Epithelial
    - Neoplasia
      - Sialocele
      - 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

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

Epithelial Cell Tumors

Adenoma: Uniform cells. Acinar formation on far right.
Mass on the Prepuce of a Dog

Diagnosis?

Circumanal adenoma/perianal/hepatoid 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

• Not uncommon w/ 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

Abdominal Metastasis
Inflammatory or Neoplastic?
Neutrophils?
Shape of cells and nuclei?
Variability?

Transitional Cell Carcinoma

Diagnostic Tree

Mass
- Inflammatory
  - Neutrophils
    - Healthy
    - Degenerate
  - Eosinophils
  - Mixed
- Non-inflammatory
  - Neoplasia
    - Hyperplasia
    - Neoplastic
      - Cyst
      - Hematoma
      - Epithelial
      - Sialocele
    - Mesenchymal
Mesenchymal (Spindle) Cell Tumors

- Osteoma / Osteosarcoma
- Hemangioma / Hemangiosarcoma
- Peripheral nerve sheath tumor (hemangiopericytoma)

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 Tumors

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 excision with wide surgical margins and histopathological evaluation.

Cytology vs. Histopathology: Diagnosis?

Skin lump, dog

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, poorly differentiated sarcoma etc.
- Grading schemes are based on histopathology; degree of differentiation, necrosis, and mitotic index (some use other criteria)
Canine Soft Tissue Sarcomas

Prognostic Factors for Cutaneous and Subcutaneous Soft Tissue Sarcomas in Dogs,
Vet Pathol 48(1):73-84 (2011)

<table>
<thead>
<tr>
<th>Type</th>
<th>Tissue of Origin</th>
<th>Phenotype</th>
<th>Histologic Hallmarks</th>
<th>Typical Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosarcoma</td>
<td>Fibrous tissue</td>
<td>Fibroblastic</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>SMA, vimentin, CD90</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>Fibrous tissue</td>
<td>Fibroblastic</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>CD34, CD56, CD90</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>Fat</td>
<td>Lipoblastic</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>CD34, CD56</td>
</tr>
<tr>
<td>Perivascular wall tumors</td>
<td>Perivascular wall</td>
<td>Perivascular, smooth muscle</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>CD34, CD56</td>
</tr>
<tr>
<td>Schwann cell sarcoma</td>
<td>Schwann cell</td>
<td>Schwannian</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>S100, GFAP</td>
</tr>
<tr>
<td>Pleomorphic sarcoma</td>
<td>Pleomorphic</td>
<td>Pleomorphous</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>SMA, vimentin</td>
</tr>
<tr>
<td>Malignant fibrous histiocytoma</td>
<td>Malignant fibrous</td>
<td>Fibroblastic</td>
<td>Irregular bundles, branching, pseudoneoplastic</td>
<td>SMA, vimentin</td>
</tr>
</tbody>
</table>

Table 1. Grade system for cutaneous and subcutaneous soft tissue sarcoma in dogs

<table>
<thead>
<tr>
<th>Grade</th>
<th>Differentiation</th>
<th>Mitotic Score</th>
<th>Necrosis</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well differentiated</td>
<td>0-9</td>
<td>0-5</td>
<td>Favorable</td>
</tr>
<tr>
<td>2</td>
<td>Well differentiated</td>
<td>10-19</td>
<td>6-15</td>
<td>Fairly favorable</td>
</tr>
<tr>
<td>3</td>
<td>Poorly differentiated</td>
<td>&gt;19</td>
<td>&gt;16</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Table 2. Grading system for cutaneous and subcutaneous soft tissue sarcoma in dogs

*Note: Combination of differentiation, mitotic, and necrosis scores.*
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

Canine Soft Tissue Sarcomas

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastasis</td>
<td>Rare</td>
<td>Rare-infrequent</td>
<td>Greatest propensity for metastasis</td>
</tr>
<tr>
<td>Recurrence</td>
<td>Infrequent frequency</td>
<td>Intermediate frequency</td>
<td>Likely</td>
</tr>
<tr>
<td>&quot;Close&quot; margins*</td>
<td>Rare</td>
<td>Rare</td>
<td>Minority of cases</td>
</tr>
</tbody>
</table>

* Precise estimates for rates of metastasis and recurrence, as related to histologic grade and completeness of surgical margins, have not been reported.
** Distance between surgically created tissue edge and neoplastic cells is less than 3 mm thickness or surgical margins do not contain normal tissue outside the pseudocapsule.
*** Distance between surgically created tissue edge and neoplastic cells is at least

Radiograph of a Cat
Cat

This is not osteomyelitis, as there are no neutrophils.
Culture not needed.
Osteosarcoma most likely

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)
Lytic Bone Lesion
Differential Dx and how do you decide?

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
  - Monoclonal 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

Agenda

• Overview:
  – Advantages
  – Limitations
• Techniques
• Interpretation
• Description
• Case examples

Cytology Description

• Every good cytology description should include the following:
  – Quality
  – Cellularity (usually only nucleated cells)
  – Predominant cell type description (shape, arrangement, nuclei, cytoplasm, cell borders, features of malignancy)
  – Important organism description (including SIZE)
  – Other cell types present
  – Background (fluid, peripheral blood, matrix)
  – Contaminants
  – IMPRESSION!
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.

Differences with Histo Description

• Color descriptors (pink vs. eosinophilic)
• Mitotic figures (presence vs. rate)
• More detail on description of cellular features (chromatin patterns, etc.)

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 rubicytes and metarubicytes (maturation 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!

Cytology Case Studies

What is your diagnosis?

Neutrophils? Are cells individualized or organized? Shape of cells? Shape of nucleus? Variability?
Mass in perineum of an adult bull terrier bitch

The aspirate at right contains...... Neutrophils? What else?

Mass in perineum of adult bull terrier bitch

Mast cell tumor
Round cells with central nuclei and metachromatic intracytoplasmic granules

Mass on ear of an 18-month-old dog

First step....Neutrophils? What is the primary nucleated cell?
Round cells with round nuclei & absence of cytoplasmic granules.
Cells resemble those found in TVT, but do not have discrete cytoplasmic vacuoles and do not contain large nucleoli.
The lesion is located on the ear, NOT the genitalia or mucous membrane!

Diagnosis?
**Mass on ear of an 18-month-old dog**

**Canine cutaneous histiocytoma**

Common benign skin tumor of young dogs; often regresses spontaneously.
Tumor cells are round with pale blue cytoplasm. Sometimes reniform (kidney shaped) nuclei.
Resemble cells of TVT, but do not possess discrete cytoplasmic vacuoles and do not contain large nucleoli.

---

**Mass on ventral neck of an adult dog**

**Inflammatory? What is the primary nucleated cell?**

---

**Mass on ventral neck of an adult dog**

**Malignant melanoma**

Large cells with multiple, green/black intracytoplasmic granules.
Similar granules from ruptured cells are scattered throughout background.
Naked nuclei are from ruptured tumor cells.
Fine needle aspirate from lytic lesion shown below right in radius of a dog

What is your differential diagnosis?

Osteosarcoma

**Multiple myeloma**

Lytic foci in bone; increased serum protein; monoclonal gamopathy; Bence-Jones proteinuria; and hypercalcemia (10% of cases; poor prognosis)
Deep-seated focal mass in neck of adult dog


Deep-seated focal mass upper neck region dog

Thyroid carcinoma

Clusters of epithelial cells associated w/ pink secretory material (above left). Occasional acini (above right).
Some cells have bright turquoise granules; probably tyrosinase and is a feature of thyroid tumors.
Although tumor appears benign cytologically, thyroid carcinomas in dogs are usually highly malignant.

Fine needle aspirate from a lump on the face of a dog

Predominate cell type?
Other cell types?
Interpretation?
Fine needle aspirate from a lump on the face of a dog

Predominate cell type?
Other cell types?
Interpretation?

Granulomatous inflammation with numerous neutrophils and moderate numbers of macrophages. No etiology is evident, but suspect higher order bacteria or fungus.

Aspirate of a mass from the nose of a horse

Interpretation?
Aspirate of mass from nose of horse
Numerous macrophages and neutrophils (pyogranulomatous inflammation) with intra- and extracellular septate fungal hyphae

Needle aspirate from face of a dog
Round cell tumor
- Differentials:
  - Histiocytoma
  - Plasma cell tumor
  - TVT
  - Lymphoma
  - Mast cell tumor

My interpretation: Plasma cell tumor – eccentric nuclei; perinuclear hoff; variation in cellular/nuclear size; chromatin pattern; no reniform nuclei

Aspirate from mass on dorsal neck of cat
Interpretation?
**Aspirate from mass on dorsal neck of cat**

- Densely cellular with clusters (rafts) of uniform-sized & shaped epithelial cells
- Scant deep blue cytoplasm; spindloid features in some cells – suggestive of basal cell layer

**Epithelial cell proliferation or neoplasm**

- Cell features and arrangement suggestive of feline basal cell tumor; occasionally, these tumors contain melanin pigment

---

**Abdominal fluid from a horse**

**Interpretation?**

- Numerous degenerate neutrophils & macrophages with intracellular bacteria
- Note toxic changes in PMNs
- Septic exudate with bacteria
Abdominal aspirate from dog with ascites

Interpretation?

+ 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 w/ carcinoma

Aspirate: Skin mass on neck; older adult dog

Interpretation?
Aspirate: Skin mass on neck; older adult dog

Interpretation: Basal cell tumor / Trichoblastoma

Dog digit: Skin impression

Interpretation?

Dog digit: Skin impression

Interpretation: Hyperkeratosis w/ Malassezia yeasts
Aspirate: Fluctuant mass, upper neck, adult dog

Interpretation?

Aspirate: Fluctuant mass, upper neck, adult dog

Interpretation: Salivary mucocele

Aspirate: Hard mass over tibia adult dog

Interpretation?
Aspirate: Hard mass over tibia adult dog

Interpretation: Sarcoma, favor osteosarcoma

Aspirate: Skin mass on head young dog

Interpretation?

Aspirate: Skin mass on head young dog

Interpretation: Cutaneous histiocytoma; reniform nucleus & lymphocytes?
Aspirate: Sub Q mass on limb of older dog

Interpretation?

Aspirate: Sub Q mass on limb of older dog

Interpretation: Spindle cell neoplasm, favor PNST

Aspirate: Mass dorsal lumbosacrum, male dog

Interpretation?
Aspirate: Mass dorsal lumbosacrum male dog
Interpretation: Hepatoid (perianal) adenoma

Aspirate: Sub Q mass thorax older female dog
Interpretation?:

Aspirate: Sub Q mass thorax older female dog
Interpretation: Lipoma
Aspirate: Nodular skin mass male working dog

Interpretation: Infundibular keratinizing acanthoma