Clinical Pathology: "Through the Looking Glass"

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Introduction

This presentation will concentrate on the avian test samples that can be seen through microscopic examination. Sample collection techniques will be discussed only in the context of possible interpretation complications. Unless otherwise noted, Wright's stain cytologies form the basis of the cell descriptions.

Cytodiagnostics

Cytology is the study of individual cells without regard to architectural patterns. It provides a simple diagnostic test to allow rapid disease management. The samples offer the greatest value when they are collected and immediately examined. This can be done during the initial contact with the patient. Armed with the findings of the physical examination, history, and cytological findings, a practitioner can make a presumptive or differential diagnosis list. This preliminary information gives the clinician the ability to immediately start appropriate therapeutics and/or request further diagnostic testing. With avian patients, quick intervention in the disease process means an improved prognosis.

The areas that favor cytological examination include: aspirations from external masses, enlarged organs such as the liver, and swollen joints; washings or fluids from the ingluvies, proventriculus, infraorbital sinus, trachea, and coelomic cavity; and impression or exfoliative cytology of open lesions, the cornea, cloaca, and oral cavity. Fecal material and urine can also be examined cytologically. Cytological evaluation is valuable in the examination of blood tissue and bone marrow. If an individual case proceeds to the necropsy table, cytological examination of selected organs or fluids may give the clues needed to attempt therapy of contact birds or provide some answer for the client as to the cause of death.

Cell Types[1,2]

The cells found in cytology samples are classified as hemic, epithelial, mesenchymal, and nervous tissue. Blood and other hematopoietic tissues such as bone marrow, liver, and spleen have hemic cells. These cells can be an important part of other tissue samples.

Epithelial cells tend to exfoliate easily. They can be found in sheets or clusters. Epithelial tissue tends to display a high cellularity. Depending on the location, the cell shapes vary from polygonal (the squamous epithelial cell), spherical, cuboidal, and columnar. Secretory epithelium may have cells with cytoplasmic granules.

Connective tissue (mesenchymal) exfoliates poorly. These cells occur singularly, appearing elongated and spindle-shaped. The fibroblast is the most common cell of this type.

Nervous tissue cells are very rare. They are deeply basophilic, stellate cells.

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Categories of Cell Reaction[1,2,3]

The categories include inflammation, benign neoplasia or hyperplasia, malignant neoplasia, and normal cytology. Normal cytology is discussed by locations. Remember, exfoliative cytology provides only an “impression” of the disease process!

Inflammation

Tissue inflammation can be caused by living organisms (bacteria, fungus, and parasites), trauma, thermal or chemical agents, and neoplasia. The cell types involved include heterophils, plasma cells, lymphocytes, monocytes, and macrophages. Eosinophils are rare or difficult cells to detect in avian inflammatory processes. Three separate degrees of inflammation can be detected.

Acute or heterophilic inflammation is characterized by heterophils comprising more than 70% of the cells. Usually, these heterophils degenerate because of exposure to a toxic microenvironment. Microbial toxins are the most common agents. Degenerative heterophils appear with increased cytoplasmic basophilia, vacuolization, and degranulation. The nucleus may be undergoing karyolysis (swollen, with a poorly defined, homogenous pink chromatin pattern) or nuclear fragmentation (karyorrhexis). A septic heterophilic inflammatory process shows bacterial phagocytosis.

Chronic active (subacute) or mixed-cell inflammation is the most common type of inflammation. Within a few hours of the tissue insult, macrophages migrate to the area. The heterophils constitute only 50% of the cell population; the rest are monocytes, macrophages, lymphocytes, and plasma cells. The heterophils are generally less degenerative than in the acute process.

The chronic or macrophagic inflammation presents with greater than 50% of the cell population consisting of monocytes, macrophages, lymphocytes, and plasma cells. Common causes include foreign body reaction, mycobacterium, chlamydia, fungi, and cutaneous xanthomatosis.

Hyperplasia and Benign Neoplasia

It is not possible to differentiate a hyperplastic reaction from a benign neoplasia by cytology. With both processes, immature cells with increased cytoplasmic basophilia and pale vesicular nuclei will be evident. The cells show a uniform nucleus-to-cytoplasm ratio. Multinucleation and an increased mitotic index may be apparent. Tissue hyperplasia results from cellular injury or chronic stimulation. Examples of hyperplasia in birds include the cell proliferations that occur adjacent to chronic inflammatory lesions, thyroid hyperplasia, and squamous cell hyperplasia from vitamin A deficiency. The lipoma is a common benign neoplasm.

Malignant Neoplasia

The cells of a malignant neoplasm are a polymorphic population. They show nuclear anisocytosis, multinucleation, variable shapes and molding. The nucleoli are abnormally large, greater than 1/3 of the nuclear diameter. The chromatin and the nuclear membrane are irregular. Usually a high mitotic index with abnormal mitotic figures appears. The cell population has variations in nucleus-to-cytoplasm ratios. Another characteristic of neoplasia is hemorrhage in the area without a history of trauma. An example would be an ovarian adenocarcinoma with abdominal hemorrhage.

Carcinomas are epithelial in origin. They exfoliate well and in aggregates. The cells are round to polyhedral. Sarcomas are mesenchymal tumors. They provide a poor cell sample that usually rests within an extracellular matrix. The cells are spindle-shaped. Discrete cell or round cell tumors are

highly cellular. The cells are round and exfoliate as individuals. An example in avians is a lymphoid neoplasia.

Excisional biopsy and histopathology usually provide greater diagnostic information in solid tumor masses than exfoliative cytology.

Cytology of Effusions[1,2,3,4]

Fluid accumulations in the peritoneal cavity classify as transudate, modified transudate, exudate, malignant, and hemorrhagic. To collect the sample, do a surgical prep of the ventral abdomen. Using a 21 to 25 gauge needle, enter the body cavity on the ventral midline, just distal to the point of the keel. Direct the needle to the right side to avoid the ventriculus on the left. Beware of an enlarged liver with fluid accumulation. The point of entry should be more distal in these cases. Fluid accumulation will collapse the abdominal airsacs, providing easy location of the peritoneal cavity.

Cells of the abdominal cavity include mesothelial cells and macrophages. Normal mesothelial cells are flat, polygonal, and have a centrally positioned round or oval nucleus. They are found both as single cells and in clusters. The cytoplasm is homogenous and weakly basophilic. Reactive mesothelial cells are more cuboidal. The cytoplasmic margins may appear scalloped or show villus-like eosinophilic margins. Multinucleation, cytoplasmic vacuolation, and mitotic activity may also be found. Reactive cells appear after serosal irritation or chronic fluid accumulation. Macrophages are large cells. The cytoplasm may show phagocytic vacuoles and occasionally foreign material.

Transudates form after a change in oncotic pressure or from a circulatory disturbance. Some examples include cardiac insufficiency, hepatic cirrhosis, and severe hypoproteinemia. Avians would probably die before developing a hypoproteinemia severe enough to form a transudate. The fluid is colorless to pale straw color and clear to slightly turbid. It is characterized by low cellularity, low specific gravity (<1.020), and low total protein (<3.0 g/dl). Transudates do not clot. The cell population includes leukocytes and reactive mesothelial cells.

Modified transudates are long-standing transudates. They show a higher cellularity and protein than a transudate. The cells typically found are macrophages, monocytes, and reactive mesothelial cells. Mynah birds with hemochromatosis frequently develop transudates or modified transudates, as do psittacines with chronic liver failure.

Exudative effusions have a high cellularity and are viscous. The specific gravity is >1.020 and total protein >3.0 g/dl. These fluids frequently have a foul odor and will clot. The cellular characteristics vary. Acute exudative effusions have primarily heterophils with a heavy granular background. Septic effusions present with degenerative heterophils and intracellular bacteria. In chronic effusions there are more macrophages, lymphocytes, and plasma cells. Mott cells may also be present. These are plasma cells with Russell bodies that contain immunoglobin secretory products. The avian viral serositis syndrome is frequently associated with a clear, yellow exudate that clots when exposed to air. Other causes include septic peritonitis, egg yolk peritonitis, and some abdominal malignancies.

Hemorrhagic effusions look like peripheral blood without thrombocytes, unless the sample was contaminated with peripheral blood when collecting. A chronic or resolving hemorrhagic effusion shows erythrophagocytosis. Hemorrhagic effusions can occur with trauma and rupture of an internal organ or rupture/necrosis of a tumor mass.

Malignant effusions can have characteristics of hemorrhagic or exudative effusions. Neoplastic cells will be evident, as well.

Cytology of Upper alimentary tract; Oral, esophagus, and ingluvies[1,2,5]

The normal cytology of the oral cavity is cornified squamous epithelium. The esophagus and crop are lined with noncornified, stratified squamous epithelium. It is normal to see extracellular bacteria and an occasional yeast organism. The bacteria frequently occur on the surface of squamous epithelial cells. *Alysiella filiformis* can be confused with a pathogenic organism, as it appears in unbranched, ribbon-like chains. This is a normal gram-negative bacterial inhabitant. A large cocci, *Sarcinia*, can be confused with yeast organisms. The author has observed that finches and canaries generally have very few bacterial organisms present in the oral cavity.

Common oral lesions in avians include septic stomatitis, candidiasis, trichomoniasis, and squamous cell hyperplasia. In septic stomatitis, expect to find inflammatory cells and bacterial phagocytosis. In psittacines, the gram-negative bacteria are more often associated with disease. Pasteurella can be identified on a gram stain from its bipolar staining. Spirochetes are easily identified; in cockatiels they appear to be associated with clinical disease. In suspected cases of candidiasis, pay attention to inflammatory cells and increased numbers of organisms (>1/hpf). Candida appears as a narrow-based budding yeast. Hypha formation indicates tissue invasion. Trichomoniasis may be observed with Wright's stained smears as basophilic, piriform cells with flagella. It is better diagnosed, however, with a wet mount preparation. Squamous cell hyperplasia from vitamin A deficiencies present with sheets or aggregates of cornified squamous epithelial cells. Usually little background debris exists in the early stages. The condition can progress to a septic stage, with secondary bacterial infections.

These same lesions can also occur in the esophagus and ingluvies. In acute cases of ingluvitis, a fairly uniform population of bacteria will be found with few inflammatory cells. "Megabacteria" has occasionally been seen on crop cytology.

Cytology of the Cloaca[1,2,5]

The normal cloaca is lined with simple columnar epithelium and lymphatic tissue. The vent has cornified, stratified squamous epithelium. It is common to find a variety of extra-cellular bacteria, amorphous debris and urate crystals. Septic processes, giardia, and capillaria can be identified with cloacal cytology. Papillomatous disease will not have any distinguishing characteristics on exfoliative cytology. Excisional biopsy is the preferred method of diagnosis. It is possible to identify avian tuberculosis from a cloacal or fecal sample. Psittacines intermittently shed the acid-fast bacteria that will appear as "ghost" cells with a Wright's stain, prompting further diagnostic staining. "Megabacteria" has been identified through cytology of fecal samples.

Cytology of the Upper Respiratory Tract[1,2,5]

The nasal and infraorbital sinus are lined with nonkeratinizing, stratified squamous epithelium. The sinus should have few bacteria. Pseudostratified, ciliated columnar epithelium with goblet cells are found in the trachea and primary bronchi. Goblet cells are columnar, lack cilia, and have abundant cytoplasm with vacuoles and eosinophilic granulation. The nucleus is large, round to oval, and eccentric in location.

Bacterial sinusitis, which is usually secondary to a predisposing nutritional problem, will show a septic, heterophilic or mixed-cell reaction. Chlamydial sinus infections often have a mixed-cell to macrophagic inflammation. Rarely has staining for chlamydia organisms in the upper respiratory exudate been productive in this author's experience. Tracheal washes and brush cytology have identified fungal infections such as aspergillosis. The author has observed an acute heterophilic inflammatory reaction in aspergillus cases of the sinuses and trachea before cytology with fungal hypha or culture could confirm the disease. As the fungal disease progresses, a mixed-cell to

macrophagic inflammatory response occurs.

Cytology of the Lower Respiratory Tract[1,2,5]

The lung, air capillaries, and air sacs are lined with simple squamous epithelium. The air sacs are poorly cellular, producing the occasional noncornified epithelial cell on cytology.

With airsacculitis and pneumonia an increase takes place in the numbers of inflammatory cells and background material. Chronic chlamydia and fungal infections show a mononuclear leukocyte response with macrophages and plasma cells. Imprints of avian lung tissue have been diagnostic for atoxoplasma in canaries.

Cytology of the Skin[1,2,5]

Skin cytology consists of squames (anucleated, cornified squamous epithelial cells), extracellular bacteria, and a variable amount of background debris. Pathogenic bacteria will be found within the leukocytes. Extracellular bacteria are nonpathogenic and will appear on the surface of the squamous epithelial cells.

Knemidokoptes mites, common on chickens and budgies, seldom create an inflammatory reaction. Cytology of the affected skin, cere, feet, legs or vent, will show hyperkeratosis and occasional mites. The cutaneous form of avian pox produces papules or raised, scab-like lesions around the eyes, beak, nares, tibiotarsus or feet. Cytological examination shows swollen squamous epithelial cells with one or more large cytoplasmic vacuoles (Bollinger bodies) that contain tiny, round, pale eosinophilic inclusions (Borrel bodies). Cutaneous xanthomatosis lesions appear as discrete nodules or diffuse thickening of the skin. The skin is yellow, friable, and featherless. Aspiration or impression cytology of a cut surface reveals excessive lipid material and a macrophagic inflammatory response. There will be numerous highly vacuolated macrophages, multinucleated giant cells, and the characteristic cholesterol crystals. Subcutaneous lipomas that are commonly found on the ventral sternal area will have fat cells (lipocytes) on cytology. A greasy appearance to the slide before staining is a consistent finding. These cells have abundant foamy cytoplasm with large vacuoles. Cytology of a cutaneous lymphosarcoma will show a marked number of lymphoid cells, the majority being immature lymphocytes. Cytology should be performed on feather cysts. More chronic lesions may develop a septic, mixed-cell inflammation that could prompt the use of antibiotics. In the early stages, more hemorrhage is evident.

Cytology of the Conjunctiva and Cornea[1,2]

The normal conjunctival mucosa is stratified columnar epithelium. Scrapings usually produce a poorly cellular sample. Conjunctival goblet cells can be found and are associated with many mucus strands. The cornea is stratified squamous epithelium, and also delivers poorly cellular samples.

In conjunctivitis and keratoconjunctivitis, inflammatory cells and an increased number of epithelial cells containing pigment granules appear. The cytology of chronic irritation or hypovitaminosis A show cornified squamous epithelial cells. Look for keratinization of conjunctival or corneal epithelium.

Cytology of Synovial Fluid[1,2,5]

Normal synovial fluid in avians has insufficient volume for sampling. The fluid contains mucopolysaccharides (hyaluronic acid), macrophages, a few leukocytes, and synovial lining cells. On cytology, a poorly cellular sample displays small and large mononuclear cells and a granular

background.

An increased number of heterophils, abnormal color, and a decrease in viscosity and mucin characterizes inflammatory lesions. The decrease viscosity is from a decrease in the hyaluronate content and the dilution of the synovial fluid by the effusion. If there is erosion of the articular cartilage, multinucleated osteoclasts or spindle-shaped fibroblasts will appear. Septic arthritis is defined by the heterophils and bacteria. In trauma red blood cells and heterophils are identified with an occasional erythrophagocytosis. Cytology of articular gout reveals the needle-shaped crystals of the urates.

Cytology of Internal Organs[1,2]

Impression cytology of various internal organs is a useful practice. This author has been able to quickly reach a definitive diagnosis prior to the histopathology report in a fair number of necropsy cases. Impression cytology identifies the source of biopsy samples taken during surgery. In avian patients suffering diffuse pathology (such as egg yolk peritonitis, visceral gout, metastatic tumors), or severe changes to internal organs, such as liver cirrhosis, this author has been able to confirm that the appropriate tissue was submitted for histopathology.

The normal liver should primarily contain hepatocytes with a variable amount of hemic cells. Hepatocytes are large cells, uniform in appearance, with round to oval nuclei that are slightly eccentric in location, and with one or two prominent nucleoli. The cytoplasm is abundant, basophilic, and granular. The cells may exfoliate individually or in groups. Macrophages may contain iron pigment, which appears dark blue-black with a Wright’s stain.

Inflammatory lesions of the liver will show an increase in mature heterophils, macrophages, and plasma cells. Avian tuberculosis and chlamydia will induce an increased macrophage, lymphocyte, and plasma cell reaction (macrophagic inflammation). Special stains can identify the etiologic organism. Hepatic lipidosis cytology contains swollen hepatocytes with cytoplasmic vacuolation. Tumor cells can be identified in neoplasia of the liver. Occasionally, parasites will appear on a liver impression cytology.

The normal spleen has a marked number of hemic cells. Chlamydia and excessive iron deposition are readily identified with special stains of splenic cytology.

The kidney is normally highly cellular. Excessive urate deposition, infections, and tumors can be identified by cytology.

Urinalysis[6,7,8]

Urinalysis remains a poorly understood diagnostic test in avians. Bird species are uricotelic and secrete a cream-colored, thick mucoid material that contains uric acid; usually in the insoluble form. The excretion of uric acid is largely independent of tubular water resorption so the concentration can become very high with a low urine flow rate. Urine production is strongly influenced by the tubular resorption of water. With diuresis, very little water may be absorbed, whereas with dehydration or low rate of urine flow, 99% is reabsorbed. Under certain conditions birds will produce a thin, watery urine described as polyuria. Common causes of polyuria include excitement, intake of large amounts of fluids (water, fruits, and vegetables), renal disease, diabetes, sepsis, some medications, liver disease, and impending egg laying.

Because of the structure of the avian kidneys and the inability of uric acid to contribute to a concentration gradient, birds have limited ability to concentrate urine. It is important to remember that

the common urine measurement, specific gravity, provides only an estimate of osmolality. In mammals, the ratio of urine to plasma osmolality measures the ability of the kidney to concentrate. In the bird, specific gravity is influenced by both hydration and osmolality. The specific gravity of avian urine from the ureters ranges from 1.0018 to 1.015.

In personal experience, only the dipstick values for blood, protein, pH, and glucose have any significance. Normal values in psittacines are pH 6-8, trace protein, glucose, and blood. Urine sediment examination has been valuable. It is important to collect the watery fraction of the urine and attempt to keep it separate from the feces. With immediate microscopic examination, the number of bacteria may be important. Increased numbers could indicate a urinary tract infection, although the contact with the digestive and reproductive tracts in the cloaca, further evaluations are required. The finding of greater than 1-2/hpf of red or white blood cells is significant. Casts, extremely rare in casework observations, indicate severe renal pathology.

Bone Marrow[2,9,10,11,12]

Bone marrow cytology in avians unfortunately is seldom used. With greater use and more widely reported results, it may prove its value, as in mammals. Some indications for collecting a bone marrow sample include a persistent non-regenerative anemia, thrombocytopenia, panleukopenia, heteropenia, and suspected cases of leukemia. Avian mycobacteriosis is more consistently diagnosed with acid-stains of bone marrow cytology as opposed to fecal samples. Bone marrow collection is contraindicated in birds too ill or anemic that further restraint endangers their lives. The common collection sites in adult psittacines include the proximal medial tibiotarsus, the widest area on the ridge of the sternum, and the femur. Most birds will require general anesthesia. Detailed descriptions and pictures are found in references number two and eleven.

The erythroid series cells are the rubriblast (proerythroblast), prorubricyte (basophilic erythroblast), basophilic rubricyte (early polychromatic erythroblast), polychromatic rubricyte (late polychromatic erythroblast), polychromatic erythrocytes (orthochromic erythroblast), and mature erythrocyte. The cells become smaller with each step in the sequence of cell maturity. Polychromatic erythrocytes are normal in the peripheral circulation in small numbers.

The granulocytic series for each cell line (heterophils, eosinophils, and basophils) follows the same pattern. The series includes, in order of maturity, the myeloblast (granuloblast), progranulocyte (promyelocyte), myelocytes (mesomyelocytes), metamyelocyte, band and mature cell. At the myelocyte stage, the cells contain half their number of mature cytoplasmic granules for the cell line. Heterophilic metamyelocytes are occasionally found in the peripheral blood. They have a round to slightly indented nucleus, and the definitive granules occupy greater than half the cytoplasm. Other granules in the cell are magenta in color. Band heterophils have a “band” nucleus that is usually obscured with granules, making them difficult to identify.

Thrombocytes develop from mononuclear precursor cells. The thromboblast is a large, round to oval ameboid cell with a narrow rim of basophilic cytoplasm. As the thrombocyte matures, the nucleus becomes more clumped and the cytoplasm becomes less basophilic, with small numbers of eosinophilic granules.

Other cells found in bone marrow are monocytes, which will travel to other body tissues to become macrophages, lymphocytes that are usually small and mature, plasma cells, and osteoblasts.

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