Pairing CBC data with bone marrow evaluation, a case based approach

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Indications for aspirating bone marrow: In small animal medicine, bone marrow is most frequently evaluated to identify or stage a neoplastic process, or to determine if intramedullary disease is contributing to changes identified in the CBC. These abnormalities include increases or decreases in cell counts, disorderly maturation, and the presence of circulating neoplastic cells. Less frequently, the bone marrow is evaluated in the search for an infectious agent.

Clinical history and timing of sample collection: Appropriate timing of sample collection and inclusion of pertinent historical data and clinical findings are essential in evaluating a hematological disease. For example, bone marrow aspiration is not indicated in cases of pre-regenerative, acute hemolytic anemias. If an anemia is nonregenerative, persistent, and cannot be explained by a concurrent disease, bone marrow evaluation is indicated. The recognition of ineffective erythropoiesis due to destruction of earlier stage of erythroid cells (precursor directed or non-regenerative immune-mediated anemias), hinges on evaluating the kinetics of the bone marrow response. Pairing enumeration of the various maturational stages in the erythroid lineage with the clinical findings, allows the pathologist to make this diagnosis. A similar construct is applied in the evaluation of myelopoiesis and thrombopoiesis. The pathologist should be informed of the duration of any reductions in hematopoietic cell lineages (cytopenias), drug history or potential exposure to toxins, and any co-morbidities. Some common drugs, such as fenbendazole, have been associated with pancytopenia secondary to intramedullary cell death.

Pairing the aspirate with a complete blood count (CBC) and core biopsy: A CBC, ideally in sample format but alternatively in numerical data, will help the clinical pathologist provide the most accurate diagnosis and useful recommendation. Pairing findings in the marrow with cell counts in the blood facilitates distinction between hypoplasia, normal cellularity, relative hyperplasia, and overt hyperplasia in each of the three lineages. The blood smear, as part of the CBC, should also be reviewed for evidence of circulating neoplastic cells. If an orderly response is evident in the peripheral blood, there may be little information to be gained by evaluating hyperplasia in the bone marrow. Bone marrow evaluation is indicated if the cause of the inflammation is suspected to be intramedullary (certain fungal infections, for example). Cellular morphology is carefully evaluated for evidence of any dysplasia, both in the bone marrow sample and the blood smear. Acquisition of a core biopsy allows for evaluation of the trabecular bone, fibrosis, and architecture of the tissue. Identification of a neoplastic infiltrate can be facilitate by immunohistochemical labeling of cells, and is often useful in identifying tumors of lymphoid and histiocytocytic origin.

A series of cases will be presented to demonstrate how numerical and morphological details provided by the CBC and blood smear evaluation inform the interpretation of bone marrow aspirates. A subset of cases will include discussion of advanced diagnostics.

Evaluation of body cavity fluids

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Parameters of body cavity fluid evaluation:
The evaluation of body cavity fluids includes evaluating the gross features of the fluid, microscopic enumeration of cells and specific cell types, as well as limited chemical testing of fluid constituents. The goal of this evaluation is ultimately to determine the underlying pathological process driving the effusive process. Gross features of the fluid include the color and clarity of the fluid. Clear fluids are more likely to be transudative in nature whereas markedly turbid fluids will likely be exudative or neoplastic in nature. A yellow-tinge to the fluid may indicate icterus, and the strawberry milkshake color of a chylous effusion is a very characteristic feature. The protein concentration is most frequently approximated by measuring the refractive index of the fluid. Nucleated cell counts are commonly determined using a hematology analyzer that employs flow cytometric methodology, or an impedence based cell counter. The cell counts should always be compared to microscopic review of a direct smear. This helps ensure that particles are not being counted as cells, and to that cellular clots are not trapping the cells and causing a falsely low count.

Classification of effusions:
The terminology applied to classify effusions can be a subject of debate. The term exudate is universally applied to fluids with a high nucleated cell count (typically above 3,000-5,000 cells/ul, depending on the laboratory) and protein concentration above 2.5 g/dL. These types of effusions form due to chemotaxis of inflammatory cells across the vessels and concurrent leakage of serum proteins. The classification of an effusion characterized by a nucleated cell count that does not strictly meet the criteria of an exudative process, but a protein concentration above 2.5 g/dL often invokes terms including “modified transudate” or “high protein transudate.” The underlying nature of these effusions often involves increased pressures involving post-sinusoidal vasculature. Transudates, effusions with low cell counts (less than 3,000 cells/ul) and a protein concentration < 2.5 g/dL, form due to decreased oncotic pressures. Pure transudates are relatively uncommon in small animals. Often, more than one process is occurring. Neoplastic and hemorrhagic effusions are termed separately.

Microscopic evaluation:
The WBC differential can help to classify the mechanism behind the effusion. For example, lymphocyte-rich effusions are often chylous in nature and associated with congestive heart failure in cats. The predominance of neutrophils should prompt a thorough inspection for infectious agents or evidence of a chemical irritant, such as bile.

Chemical analysis:
Quantification of substances that should not be present in the body cavity at a higher concentration that plasma are often helpful in identifying the underlying etiology. Examples include triglycerides, bilirubin, and creatinine.

Cytological review of common skin lesions and lymph node aspirates

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General goals of cytological evaluation of skin lesions:

Skin lesions (dermal and epidermal) should be distinguished, when possible, from those arising in the subcutaneous tissues. The goals of cytological evaluation of these samples are first to determine if a diagnostic sample was obtained, and second to determine if the evaluation of the sample by a clinical pathologist is indicated. The separation of inflammatory lesions from a neoplastic processes is the primary point of dichotomy, and can often been accomplished in clinic. When a recognizable infectious agent (bacteria consistent with a Staph or Strep) fit the clinical suspicion is found, addressing the infectious process if often prudent prior to re-sampling at a later date. If no cause for the inflammation is found, structures that are not readily identifiable are noted, or tissue cells are evident in the sample, submission of the sample is recommended. Neoplastic lesions can often be secondarily inflamed, which can confound the interpretation. This is very common in cases of squamous cell carcinomas, and extreme care must be taken not to over-interpret dysplastic changes secondary to the inflammation. These samples should always be submitted to a clinical pathologist, and may require further evaluation with histopathology. Many common adnexal tumors tend be benign. The goal of cytological evaluation should not necessarily be definitive identification of specific tumor type, but rather exclusion of a process that might warrant further evaluation prior to surgical removal (eg a mast cell tumor indicating the need for wider margins than a sebaceous adenoma). Tumors arising from cells within or near the blood vessels in the skin tend to exfoliate, and can often be diagnosed cytologically. Grading of these lesions often requires histopathological evaluation of tissue architecture and invasion.

Lymph node aspirates:

Aspiration of lymph nodes is a primary modality for the diagnosis of lymphoma, evaluation for metastatic processes, and less frequently the evaluation of inflammatory conditions. If more than one lymph node is enlarged, aspiration of multiple nodes and avoidance of the mandibular lymph node, if possible, is recommended. Neoplastic lymphocytes are often readily exfoliative, but unfortunately also rupture easily. Thus, gently spearing of the aspirate is critical. The quality of the sample collected can be checked by quick-staining the sample, taking care to avoid the common pitfall of overstaining nuclear material by prolonged contact with the basophilic/azure dye in use. When in doubt, go light! It is far easier for a clinical pathologist to put an under-stained sample through their preferred staining procedure than it is to de-colorize and overstained sample. Metastatic populations can be identified in lymph node aspirates, and occasionally this can occur without identification of the primary lesion! There are some challenges in determining if low numbers of cells from a tumor are found in an aspirate as a result of their presence in the sinuses of the node, or if they have set up a neoplastic niche and are propagating the node. The evaluation of lymph node aspirates for metastasis of a mast cell tumor can be particularly challenge, as these cells are not only found in the nodes of healthy animals but also for reasons unrelated to cancer. Hypersensitivity conditions and inflammation in general can call mast cells to a lymph node, as they are part of the fibroblastic response in would healing. Infectious causes of lymphadenitis can often be identified using this modality.