Proceeding of the
ACVP Annual Meeting

Nov.17-20, 2013
Montréal, Québec, Canada

**American College
of Veterinary Pathologists**

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Historical Overview of Evidence-Based Diagnostic Cytology
Including Bone Marrow in Veterinary Medicine

Rose E. Raskin, DVM, PhD, Diplomate ACVP (Clinical Pathology)
Department of Comparative Pathobiology, Purdue University
West Lafayette, IN (rraskin@purdue.edu)

Introduction

"Evidence based medicine, whose philosophical origins extend back to mid-19th century Paris and earlier, remains a hot topic for clinicians, public health practitioners, purchasers, planners, and the public ….The practice of evidence based medicine means integrating individual clinical expertise with the best available external clinical evidence from systematic research."[1] Evidence-based cytology in a related manner "is concerned with generating a reproducible, high quality and clinically relevant test result in the field of cytology." [2] However, except for gynecologic cytology, human cytopathology poorly uses the highest levels of evidence-based approaches that include systematic review, meta-analysis, and randomized controlled trials in comparison to clinical medicine. [3]

Veterinary cytology is a discipline ripe for the incorporation of quality assessment as a recent survey of 870 responding veterinarians indicated an average of 48% cytologic samples were evaluated in-house while 52% were sent to a veterinary diagnostic laboratory for interpretation [4]. General practitioners most commonly used in-house cytologic evaluations for the diagnosis of lipomas and mast cell tumors in addition to the examination of ear, skin, and vaginal swabs or scrapings. In the same survey, more than 80% of respondents indicated as very important and ranked as highest factors, accuracy and availability of cytopathology expertise, in a decision to use cytology as a diagnostic method. Recognizing the need for quality assurance, recently updated ASVCP guidelines were published that focused on preanalytical, analytical, and postanalytical factors for cytology [5]. While these guidelines discussed assessing accuracy of cytologic interpretation, there may need to be more expanded information specific for veterinary cytologic and bone marrow specimens. An excellent discussion on evidence-based cytology in veterinary medicine cites several examples of correlation studies and updated cytologic tools. [6]

Morphologic Diagnosis – Empiricism (Observation and Experience)

Cytology in veterinary medicine became a recognized diagnostic entity in the 1970's. Dr. Victor Perman, often considered the "grandfather of veterinary cytology", frequently lectured and wrote about the art and science of the discipline beginning in the mid-1960's. In addition to review or descriptive articles, several guides or atlases developed out of the new found interest in this diagnostic tool used to recognize infectious agents and evaluate skin lesions, lymph node enlargements, and body cavity effusions in clinical practice. [7-12] Similarly with regards to bone marrow morphology, a few atlases or textbooks are available as references. [13-16] Numerous single case reports published in the "What's Your Diagnosis" section in *Veterinary Clinical Pathology* have long been a popular method to highlight unique cytologic appearances or novel

confirmatory tests. Stronger evidence than the single case report is a published series of similarly affected animals such as an evaluation of the morphologic appearance of an adnexal neoplasm or clinicopathologic features of a bone marrow neoplasm. [17,18] In addition, moderate levels of evidence are provided in prevalence studies such as the degree to which myelin is observed in cerebrospinal fluid or mast cells in the bone marrow of cases involving monocytic ehrlichiosis. [19,20] See Figure 1 to view the relative hierarchy of evidence suitable for cytologic studies.

Lately the widespread availability of internet resources such as the professional-use listserv (e.g., ASVCP) and discussion groups (e.g., Veterinary Cytology on Facebook) to view images and provide "curbside consultations" has increased greatly over the past decade. While these methods are based on the lowest level of evidence, namely expert opinion or consensus diagnosis, they still provide a valuable mechanism to enhance learning and improve accuracy.
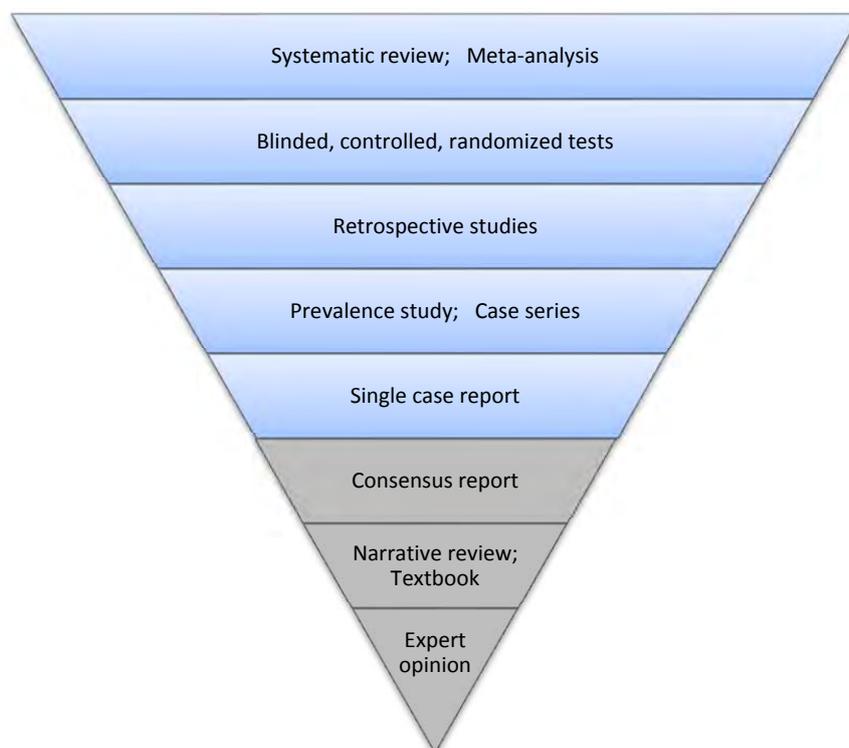

Figure 1. Hierarchy ladder shows relative value and validity for forms of evidence applicable to veterinary cytology and bone marrow samples. Grey shaded areas lack peer review and provide poor evidence.

Preanalytical and Postanalytical Factors in Cytology and Bone Marrow Examination

The area of preanalytical factors in cytology has historically received much attention and a high level of evidence-based approach through prospective studies and controlled testing. Proper collection, handling and storage, as well as staining prior to evaluation significantly affect the quality and accuracy of cytologic examinations. Recent examples of such studies include those involving collection and handling techniques [21,22], storage [23,24]; use of previously stained slide material [25,26], or type of stain procedure. [27] Preanalytical factors relative to bone marrow examination recently evaluated involve collection techniques [28,29] and site selection.[30,31]

Postanalytical factors include aspects of reporting that satisfy the needs of the clinician and provide adequate communication of the condition by the pathologist [4,32,33]. The value of stating a clear and concise interpretation cannot be overemphasized as the use of cytology has increased in the percentage of cases submitted to veterinary hospitals over time and more than half of these cases often involve neoplasia.[34]

Confirmatory Diagnostic Methods

Accuracy and reproducibility of the pathologist's diagnosis are key elements for evidence-based cytology. For cytology, the historical gold-standard has been histopathology with or without the use of special stains. However, recently this is evolving to incorporate newer ancillary diagnostic methods such as immunologic and molecular testing. The value of these ancillary studies is well-recognized in human cytopathology[35] and is expected to show similar advantages for veterinary cytology. For leukemic bone marrow conditions, the use of cytochemistry as an ancillary tool in veterinary medicine has also evolved to include immunologic and molecular techniques.[36-38] Other ancillary tests include advanced morphologic and immunologic studies using proliferation markers to assist in the recognition of neoplastic cells. [39,40]

Supportive microbiologic assays provide definitive information regarding diagnostic conclusions. [41,42] In addition, clinical outcome is necessary to confirm suspected morphologic diagnoses. This was included in a report of canine mammary tumors by evaluating postoperative outcome.[43] Another report evaluated cases one-year post cytologic-histologic examinations for neoplastic progression to add confidence to the correlation study.[44] Follow-up of clinical outcome is evaluated too infrequently in veterinary studies and more attempts to include this confirmatory evidence are needed.

Studies to support use of ancillary tests should minimize bias and involve standardized approaches with established morphologic criteria.[45-49]. Cytologic and histologic correlation studies are helpful but the specific correlation being tested needs to be well-defined. For example, those correlations examining the presence of neoplasia, inflammation, or malignancy are reasonable whereas correlations of specific neoplasms or diseases without use of ancillary testing may limit their value. Several studies [43-44,49-51] have examined these issues in an evidence-based manner using STARD (Standards for Reporting of Diagnostic Accuracy) to fully disclose the study parameters.[52]

Pathologists should have a sense about the sensitivity, specificity, predictive value, and diagnostic accuracy for masses in commonly examined areas as indicated below.

Skin and Ear Masses

A retrospective study of 292 palpable cutaneous and subcutaneous masses collected by fine needle aspiration from 242 dogs and 50 cats from 1999-2003 were compared with histopathology.[50] Cytologic samples were obtained by FNA and histopathologic samples were collected by surgical biopsy or at necropsy. The agreement between the diagnoses of neoplasia versus non-neoplasia was evaluated using histopathology as the gold standard. For diagnosing neoplasia, cytology had a sensitivity of 89.3%, a specificity of 97.9%, a positive predictive value of 99.4%, and a negative predictive value of 68.7%. Despite the limitations of obtaining satisfactory specimens, overall agreement between cytology and histopathology to determine neoplasia versus non-neoplasia for cutaneous and subcutaneous masses was 90.9% (221/243) of cases.

A study that evaluated external ear masses in cats with histologic correlation concluded that fine needle biopsy was sufficiently accurate for distinguishing inflammatory polyps from neoplasia.[53] However, histopathology confirmation was recommended for differentiation of benign proliferation and malignant neoplasia.

Bone

A retrospective study was conducted to compare the agreement between cytology and histopathology to distinguish between neoplastic, inflammation, and non-neoplastic proliferative canine bone lesions.[51] A computerized search of canine medical records identified 52 cases with sufficient diagnostic material. Cytology versus incisional (IB) and cytology vs excisional biopsy (EB) were compared pairwise for agreement. The correlation in disease process between cytology and IB was 71%, and for EB was 71%. When both histologic methods were combined and compared against cytology, there was an overall agreement of 73%. Strong agreement (92%) occurred between cytology and histology relative to the diagnosis of neoplasia. However, there was poor agreement (27%) for the combined inflammatory and non-neoplastic proliferative conditions. Cytology cellularity significantly affected rates of correlation. Cytologic diagnoses from highly cellular samples are more likely to correlate with histopathology than those from less cellular samples.

Another study evaluated diagnostic accuracy of ultrasound-guided fine needle aspiration in agreement with histopathology.[54] Aspirations from 32 diagnostic aggressive appendicular bone lesions having cortical lysis or periosteal proliferation were evaluated by cytology and histology. Cytologic diagnosis of sarcoma lesions were further examined following alkaline phosphatase staining. Cytology indicated sarcoma, with a sensitivity of 97% and a specificity of 100%; however this may be skewed by exclusion of the non-diagnostic samples. When a diagnosis of sarcoma was made on cytology, alkaline phosphatase staining suggested osteosarcoma, sensitivity was 100%, specificity 67%, positive predictive value 96%, and negative predictive value 100%.

Gastrointestinal Tract

The level of agreement between fine-needle aspirates and impression smears of gastrointestinal tract tumors in dogs and cats relative to a reference method of histopathology was evaluated in a retrospective case series. [55] Animals involved 38 dogs and 44 cats with histologically confirmed gastrointestinal tract tumors. Cytologic examination of fine-needle aspirates (n = 67) or impression smears (31) were compared with the histologic diagnosis, and extent of agreement was classified as complete, partial, none, or undetermined. Complete agreement was defined as agreement in regard to both cell lineage and cell type. Partial agreement was defined as agreement in regard to cell lineage, but a lack of agreement in regard to cell type. No agreement was defined as a lack of agreement in regard to cell lineage or pathologic process. Extent of agreement was classified as undetermined if the cytologic specimen was unsatisfactory due to hypocellularity, hemodilution, or necrosis. For 48 of the 67 (72%) fine-needle aspirates, there was complete or partial agreement between the cytologic and histologic diagnoses. For 29 of the 31 (94%) impression smears, there was complete agreement between the cytologic and histologic diagnoses, and for 2 (6%), there was partial agreement. A higher proportion of samples with complete, complete or partial agreement were significantly higher for impression smears than for fine-needle aspirates. The agreement between results of cytologic examination of impression smears and the histologic diagnosis appeared higher than for aspirate smears demonstrating that histologic correlation may be influenced significantly by the method of cytologic collection for gastrointestinal tumors.

Spleen

The diagnostic accuracy between ultrasound-guided fine-needle aspirates of splenic lesions was compared to histologic evaluation.[56] This retrospective study involved splenic conditions that were benign, neoplastic, or inflammatory from 31 dogs and cats. Cytologic samples were obtained by fine-needle aspiration, and histologic specimens were obtained via necropsy, surgical biopsy, or ultrasound-guided cutting-needle biopsy. The cytologic diagnosis corresponded to the histologic diagnosis in 19 of 31 (61.3%) cases. When broken down by histologic diagnosis, 11 of 14 (78.5%) animals with non-neoplastic conditions (reactive lymphoid hyperplasia or extramedullary hematopoiesis) and 8 of 15 (53%) neoplastic conditions agreed between cytologic and histologic evaluations. Lack of agreement occurred in 5 of 31 (16.1%). Histologic evaluation of tissue architecture was required to distinguish between reactive and neoplastic conditions in 7 of 31 (22.6%) cases as the results were equivocal. The case selection in this study was too small to significantly evaluate the agreement within specific splenic neoplasms, reactive lymphoid hyperplasia, extramedullary hematopoiesis, or inflammatory cases. It may be concluded that the moderate agreement between cytology and histology in these cases may reflect the need to evaluate tissue architecture.

Mammary Tissue

A recent study evaluated 90 canine mammary neoplasms to determine diagnostic accuracy of fine-needle aspiration cytology compared with histopathology.[57] Three aspirations were performed on each mammary gland mass using a 22-gauge needle attached to a 5-ml syringe before the mammary glands were surgically excised and submitted for histopathologic

examination. Twenty-five (27.7%) of 90 samples were classified as inadequate for cytologic diagnosis related to paucicellularity, significant blood contamination, inflammatory cells, and indistinguishable morphology. The diagnostic accuracy, sensitivity and specificity of cytologic examination for diagnosing malignancy were 96.5%, 96.2% and 100%, respectively. Multiple and larger aspirations appears to improve diagnostic accuracy in comparison to previous studies using only one 3ml syringe aspiration.

Future Implications – Quality Assurance

In a recent review on evidence-based medicine for veterinarians, a number of obstacles are presented such as a lack of high quality patient-centered research, the need for basic understanding of clinical epidemiology by veterinarians, the absence of adequate searching techniques and accessibility to scientific databases and the inadequacy of evidence-base medicine tools that can be applied to the busy daily practice of veterinarians. [58] Studies are cited related to the difficulty faced by physicians to keep on top of the information explosion. To remain fully informed, physicians should read 19 articles per day, 365 days per year, which is in marked contrast to the actual time (< 1 hour) per week available for reading. Evidence shows that physicians prefer to rely on the experience of professional colleagues than to search the literature. [59]

In conducting a literature review many resources are available; however, few veterinary-specific databases are readily available to those outside academic or industrial settings. Some popular internet resources include Veterinary Information Network and International Veterinary Information Service. General medical databases include PubMed MEDLINE, CABDirect, Google Scholar, and several publisher-specific search engines. Highest level of evidence-based research includes systematic reviews and quantitative analysis which require extensive analysis of previously well-designed studies to form specific conclusions about prognosis, instrumentation or techniques, and confirmatory tests for diagnosis. Pathology training programs should emphasize familiarity with library resources plus provide guidance in the use of search strategies so to include terms such as accuracy, sensitivity, specificity, along with MeSH (medical subject headings). In addition to encouragement to perform research or correlative cytologic and histologic studies,[60] pathology trainees must be sensitized to the need for an evidence-based approach to cytologic examination which may include formal joint conferences between clinicians and pathologists. In this format, all parties share in the learning experience, distribution of information, and need for continuous appraisal of the decision-making process and outcomes to improve proficiency and clinical significance. Furthermore to ensure improved patient care, more effort should be given to provide clinicians with clear unambiguous cytologic diagnoses. This means limiting the use of popular modifiers such as possible, probable, or suspicious and state only what is known with high confidence. It is intended that in so doing, cytopathologists will no longer be viewed as "fence sitters".

Summary

Acceptance of an evidence-based pathology or quality assurance for cytologic examination must be continually supported. While this is not a new science in veterinary medicine, it may be scorned because of new terminology. Pathologists should remain skeptical of so-called known truths and vigilant to support diagnoses by scientific methods. With adherence to evidence-based approaches cytology will remain a purposeful, minimally invasive, and quick method that can provide accurate information to clinicians.

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