CANCER TESTING: BEYOND THE BIOPSY

Kim A. Selting, DVM, MS, DACVIM (Oncology)
College of Veterinary Medicine
University of Missouri, Columbia, MO
seltingk@missouri.edu

INTRODUCTION

A standard and reliable approach to the diagnosis of a cancer case typically will lead the experienced veterinarian to an answer. Following a thorough physical examination and baseline bloodwork, fine needle aspiration of a mass will begin to distinguish a benign from malignant process. Cytology also can often categorize tumors as round/discrete cell, epithelial, or mesenchymal origin. Biopsy then usually confirms the diagnosis.

But what if this approach leads to a dead end at any one of these steps? What do you do when the dreaded "nondiagnostic sample" follows the word "results?" Sometimes the history, physical examination, cytology and biopsy all support a diagnosis, but the histologic changes are not definitive. Do you treat based on an educated guess? Sometimes. Or do you take one more step for that peace of mind in your diagnosis?

In addition, some of the biomarkers to be discussed may help monitor response to therapy, and confirm success of therapy.

There are several advanced tests developed to confirm or diagnose cancer in animals. This talk will review several commercially available tests and introduce tests on the horizon.

COMMERCIALLY AVAILABLE TESTS

ALP STAINING (confirm diagnosis of osteosarcoma)
Osteosarcoma (OSA) is a common tumor in dogs, with approximately 10,000 new cases diagnosed each year. Radiographic changes can be reliable when characteristic, sometimes more so than a biopsy. It can be difficult to confirm a diagnosis on histology because samples are small and reactive bone may predominate, or a chondroid or fibrous component make differentiating OSA from chondrosarcoma or fibrosarcoma, respectively. Alkaline phosphatase (ALP) is a hydrolytic enzyme present in multiple tissues and cell types, including osteoblasts. Neoplastic osseous tissue that is producing bone should stain positive for ALP. Cytology of the bone lesion can be strongly supportive of OSA, and if suspicious cells stain positively for ALP this can provide enough evidence of OSA to make treatment decisions. This test is available at many laboratories and is very sensitive for OSA, with a sensitivity of 100%, and specificity of 89%.

VBTA (screen for transitional cell carcinoma)
The Veterinary Bladder Tumor Antigen test is commercially available through Antech and other laboratories, or the kit can be ordered through Polymedco (www.vetbta.com). The basis for this test is antibody-mediated detection on a rapid latex agglutination dipstick for tumor-associated glycoproteins. These glycoproteins are of high molecular weight and consist predominantly of basement membrane proteins, released when invasive tumors degrade the basal lamina. This is as simple as a high school litmus test. Specificity and sensitivity for TCC

are 41% and 88%, respectively. Though false positive tests can occur with hematuria, glucosuria, and pyuria, this test can serve as a simple interim step between inexpensive, noninvasive tests and more expensive or invasive tests because the negative predictive value is high. For older dogs with pollakiuria and with abnormal or suspicious epithelial cells and without hematuria, a positive VBTA should warrant an abdominal ultrasound and significantly increase the suspicion of a transitional cell carcinoma.

PETSCREEN (confirm or monitor lymphoma)
Petscreen, Inc. (www.pet-screen.com), is a proteomics-based company out of the United Kingdom focused on novel biomarkers for a variety of disease conditions. Studies by the company found 19 serum protein peaks that are significantly different between dogs with (n=87) or without (n=92) lymphoma, 2 of which correlated well with a final confirmed diagnosis of lymphoma. This test can serve to confirm a suspected or elusive diagnosis, and may be useful in monitoring response to therapy.

PARR (confirm diagnosis of lymphoma and immunophenotype)
Polymerase chain reaction for Antigen Receptor gene Rearrangement (PARR) for canine lymphoma is based on the premise that the immune system produces a highly diverse set of antigen receptors in order to respond to as many antigens as possible. When clonal expansion of a lymphocyte occurs, as is the case when cancer develops from one renegade cell, the resulting population tends to produce only one type of receptor. This cell population can be amplified using PCR and the monotony of receptors is detected as a single band on a gel, whereas inflammatory conditions or normal lymphocytes produce a ladder effect. DNA is very stable and needle aspiration slides (stained or unstained) can be sent by regular mail for testing. Even formalin-fixed slides can be used. The test is available through Colorado State University (http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx), as well as North Carolina State University (http://www.cvm.ncsu.edu/dphp/labs/clinicalimmunologylab.html) and others. This test can be used to confirm an elusive lymphoma diagnosis. CSU reports a 75% (dogs)/65% (cats) sensitivity and a >90% specificity. A minimum of 50,000 cells (total) is needed for PARR so consideration should be given prospectively to fluids sent in small volumes such as effusions or CSF. PARR can provide information both on clonality and on cell type of origin (T vs. B cell, based on primers) but is less accurate than flow cytometry.

FLOW CYTOMETRY (determine immunophenotye of lymphoproliferative neoplasia)
Immunophenotyping has traditionally been performed on formalin-fixed biopsy samples using immunohistochemical markers. However, antibodies for this process can also be applied to cells in suspension, allowing immunophenotyping to be done on fine needle aspirate samples. This is convenient for dogs with lymphoma as it does not involve anesthesia and provides prognostic information with T-cell performing worse than B-cell for multicentric lymphoma. This service is available through many laboratories including Idexx, Veterinary Diagnostics (www.vdxpathology.com), UC Davis, North Carolina State University, and Colorad.o State University. Samples must be shipped overnight on ice, in a form that will allow a single cell suspension (FNA or CSF suspended in saline, or sample in EDTA, for example). Flow cytometry correlates better with immunohistochemistry than PARR.

c-KIT STAINING AND MUTATION STATUS (mast cell tumor prognosis and treatment)
The role of the tyrosine kinase receptor c-kit in canine mast cell tumor has been elucidated and has lead to new advances in the treatment of this disease. It has been shown that dogs with

mutated c-kit, most often in the juxtamembrane domain, have a higher response rate to novel receptor tyrosine kinase inhibitors (RTKIs) than do dogs with wild type c-kit (approximately 80% vs. 50%, respectively). Although c-kit staining is often included in "mast cell tumor panels" for proliferation markers, it is poorly predictive of outcome. Rather, it confirms the presence of c-kit as a target for therapy. One exception is that pattern of staining (membranous location, cytoplasmic location or both) can correlate well with grade and thus with outcome. Because wild type c-kit can respond to RTKIs, determination of mutation status is not routinely performed by some clinicians. However, the presence of a mutation may encourage the use of an RTKI earlier or first-line in the course of treatment. Staining for c-kit via immunohistochemistry is available through several laboratories (identification of truncated protein) and some will also sequence to determine the nature of the mutation.

1. University of Missouri (http://www.cvm.missouri.edu/vpbio/index.html)
2. Michigan State University (will sequence) http://www.pathobiology.msu.edu/
3. The Animal Medical Center (http://www.amcny.org/doctor/specialty/pathology.aspx)
4. North Carolina State University (will sequence) see link above

PROLIFERATIVE INDICES (primarily for mast cell tumors, also soft tissue sarcomas)
Various combinations of the tests below are offered in panels at laboratories including the Animal Medical Center and Michigan State University as shown above. These may be helpful in cases of grade 2 mast cell tumors for which other parameters are borderline, and a decision for or against systemic chemotherapy hinges on the likelihood of metastasis. These indices have also shown promise in better characterizing the local versus systemic behavior of soft tissue sarcomas.

AgNORs Agyrophilic nucleolar organizing regions are loops of proteins associated with the nucleoli that dissociate from the nucleoli in cancer cells, spreading throughout the nucleus, and increase in number and size. AgNORs can be performed on cytologic specimens and correlate well with grade and outcome. They have been the most predictive proliferation marker in dogs with mast cell tumors.

Ki67 Ki-67 is a non-histone nuclear protein expressed throughout the cell cycle, only in cycling cells. Immunohistochemistry using the MIB-1 antibody can detect Ki-67 in tissues and this has been very helpful in determining the prognosis for tissues such as mast cell tumors and soft tissue sarcomas.

PCNA Proliferating Cell Nuclear Antigen is a non-histone protein that gradually increases in G1, peaks in S and decreases in the G2 phase of the cell cycle. Its role is to hold the DNA polymerase on to the DNA strand during transcription. PCNA has been the least useful of the markers of proliferation as it has correlated poorly with outcome and correlations have been inconsistent when present.

MVD Microvessel density, or a measure of how many blood vessels are forming in a tumor and how close together they are, has been used as a surrogate endpoint for antiangiogenesis investigations and can correlate to tumor behavior, though inconsistently.

MITOTIC INDEX As part of routine histopathology, mitotic index (number of mitotic figures per ten high power fields at 400x) is often reported. Recent studies have shown that a mitotic index greater than 5-7 in a canine mast cell tumor is associated with significantly shorter survival times. This index is evaluated in all tumor types, and a MI greater than 3 correlates with malignant behavior for melanoma of any location.

THYMIDINE KINASE (any cancer, but primarily lymphoma and hemangiosarcoma)
Unlike the proliferation indices discussed above, thymidine kinase is a soluble biomarker of proliferation which means it can be measured in the bloodstream. This enzyme is part of the salvage pathway for DNA synthesis and can reflect a population of cells in the body that is proliferating; studies have shown great promise in detecting, prognosticating, and monitoring certain cancers such as B-cell lymphoma and hemangiosarcoma. TK can be combined with c-reactive protein (CRP, a classic inflammatory marker) to detect patterns of biomarker change that are more consistent with infectious/inflammatory versus neoplastic change. Increases in these markers can be used to screen patients for occult disease and justify additional testing and imaging. More information is available at www.vdilab.com.

VITAMIN D (any cancer, heart disease, inflammatory disease)
Pet owners often place great importance on complementary and alternative therapies, and may supplement their pets without confessing to the veterinarian. For some nutritional, immunologic, and other adjunctive cancer therapy there is good scientific data to support these decisions, while for others information is scant. There is a large body of information that demonstrates low vitamin D as a risk factor for cancer, heart disease, inflammatory conditions, and more. In vitro data has shown that vitamin D can sensitize cancer cells to the effects of chemotherapy. Normal values for 25(OH)vitD (the storage form of vitamin D) can be easily assayed and supplementation considered. www.vdilab.com or Michigan State University DCPAH. Studies are ongoing to determine optimal supplementation and its benefits.

SPECIAL IMAGING
Anatomic imaging (radiographs, CT and MRI) and functional imaging (nuclear scintigraphy) have been used for many years to define the presence and limits of cancer. Newer technologies are becoming available including PET (positron emission tomography) imaging which can be linked to anatomic imaging to find the “needle in the haystack”, can better stage cancer, and characterize a physiologic process. Though facilities are uncommon, certain cases may be ideal candidates such that travel is both justifiable and attractive to the pet owner.

ON THE HORIZON

HEMANGIOSARCOMA BLOOD TEST
Research in the past few years has identified markers of hemangiosarcoma in circulating blood. Hemangiosarcoma cells were found to express both markers of stem cell origin (to identify them as neoplastic and as originating as multipotential, bone marrow-derived) and markers of lineage commitment (to identify them as hemangioblasts). These markers can be exploited to develop a blood test that may be used to confirm the diagnosis, improve our ability to diagnose cases early in the course of disease, monitor the efficacy of treatment, or monitor for relapse or metastasis. This is similar to the circulating tumor cell (CTC) test for carcinomas in people, as well as other screening or monitoring tests in people that are based on cluster of differentiation markers to identify the presence of abnormal cells (such as CD125 in ovarian cancer). This test is currently in development for commercial applications.

GENETIC SCREENING FOR CANCER RISK
Since the sequencing of the canine genome in 2005, researchers have been hard at work investigating the genetic basis of cancer, among other diseases. Cancers with a heritable component such as osteosarcoma will undoubtedly be better understood with these

investigations and it is plausible that tests for certain genes associated with the development of this and other cancers will be developed. To contribute samples from purebred dogs with cancer to be a part of the big picture of finding a cure for cancer, go to www.dogdna.org .

SELECTED REFERENCES:

Ewing J. A review and classification of bone sarcomas. Archives of surgery 4:485-533, 1922.

Barger A, Graca R, Bailey K, et al. Use of alkaline phosphatase staining to differentiate canine osteosarcoma from other vimentin-positive tumors. Vet Pathol. March 2005;42(2):161-5.

Henry CJ, et al. Evaluation of a bladder tumor antigen test as a screening test for transitional cell carcinoma of the lower urinary tract in dogs. Am J Vet Res. August 2003;64(8):1017-20.

Romansik E, Reilly CM, Kass PH, et al. Mitotic index is predictive for survival for canine Cutaneous mast cell tumors. Vet Pathol 44(3):335-341, 2007.

Von Euler HP, Rivera P, Aronsson AC, et al. Monitoring therapy in canine malignant lymphoma and leukemia with serum thymidine kinase 1 activity- evaluation of a new, fully automated non-radiometric assay. International Journal of Oncology 34:505-510, 2008.

Lamerato-Kozicki AR, Helm KM, Jubala CM, Cutter GC, Modiano JF. Canine hemangiosarcoma originates from hematopoietic precursors with potential for endothelial differentiation. *Exp Hematol* 2006 Jul;34(7):870-8.