A Practical Guide to Diagnostic Testing for Veterinary Cancer Patients

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Introduction:
Increasingly, people who own ill pets are faced with decisions regarding the performance of tests to achieve a definitive diagnosis. Increasing sophistication in veterinary medicine, coupled with high owner expectations often means more testing, greater expense, and occasionally, potentially higher risks when the tests require invasive procedures. Moreover, there are explicit or implicit promises for some tests advertised through the Internet and in social media, which might not stand up to careful scientific scrutiny. This has led to considerable confusion regarding when, if, and what tests are appropriate or recommended for use to diagnose cancer in companion animals or whether any of these tests can be used to detect cancer in the early stages or predict if a healthy dog will develop cancer. This article is intended to provide pet owners with some basic understanding of the diagnostic process – why and when testing is necessary, what are some the benefits and what are some the pitfalls. It also is meant to encourage communication with primary care veterinarians and specialists in order to maximize the potential of a team approach to health care.

Definitions:

Cancer

The National Cancer Institute defines cancer as "*a disease in which abnormal cells divide without control and are able to invade other tissues*." Cancer cells can spread to other parts of the body through the blood and lymph systems. Cancer is not just one disease; there are more than 100 different types of cancer and the main thing they have in common is uncontrolled proliferation.

The same definition applies to cancer in domestic animals. Cancer is a common ailment in our pets today: it is estimated that approximately 1 in 3 dogs will develop cancer in their lifetime. Besides the physical burden on the dog's health, this diagnosis carries an immense psychological burden on his or her human family. The best weapon against these physical and psychological ravages of cancer is information.

Diagnostic testing

A diagnostic test is "*a medical test performed to aid in the diagnosis or detection of disease*." Diagnostic tests include routine components of the physical exam, as well as more sophisticated assays that require further training to interpret and possibly specialized equipment to perform. Clinical laboratory testing is a large field onto itself.

The diagnostic utility of any test is highly dependent on the presence of robust quality control and quality assurance practices in the laboratory performing the test. A test should have known **Sensitivity**, which is the proportion of patients with the disease that test positive. It also should have a known **Specificity**, which is the proportion of patients without disease that test negative. Sensitivity and specificity describe the capability of the test to distinguish between patients with and without disease. The **Predictive value** of a test describes the probability of disease given a particular test result, and it is influenced by how

commonly the disease occurs in a specific population. Sometimes tests are evaluated based on a population that has different characteristics than the group of patients receiving the test in practice, which can mean the test does not identify disease in clinical settings in exactly the same way it did in research studies. Testing performed in the United States on human patients or using human samples is governed by federal regulatory standards under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 and with oversight from the Food and Drug Administration (FDA).

There are no comparable regulatory standards in veterinary medicine. The American Association of Veterinary Laboratory Diagnosticians, Inc. (AAVLD) provides accreditation for veterinary diagnostic laboratories based on their ability to meet established criteria, including those that define "good laboratory practices" (GLP). This accreditation is voluntary and largely limited to the State Diagnostic Laboratories with an emphasis on diseases that have agricultural impact or that might be transmissible to people. Thus, other laboratories may or may not have mechanisms in place to ensure or approximate GLP standards. In general, commercial diagnostic labs operate at or near GLP standards, but there is much variability when point-of-care testing is done.

Biomarker

A biomarker is "*a characteristic that is objectively measured and evaluated as an indicator of normal biology, pathology, or the response to treatment.*" Biomarkers can provide important information to determine risk, aid in the diagnosis of a disease, determine prognosis, or plan the treatment strategy for a patient. Biomarkers include substances or compounds that many people would consider to be "routine," for example blood sugar to monitor diabetes. They also can be highly specialized and sophisticated and apply to only a small population of patients.

Early detection of cancer

The American Cancer Society endorses a variety of tests that can help identify cancer in humans during the early stages of the disease. "Early detection saves lives," it is said, and in many cases this is absolutely true. Cancers detected early are oftentimes more curable, and since patients with early stage disease are less likely to have frailties seen in patients with advanced disease, treatments are better tolerated.

Nonetheless, some common screening tests that have been embedded as gold standards for early detection such as the Prostate Specific Antigen or PSA for prostate cancer in men and mammography for breast cancer in women have recently become shrouded in controversy. The main issue of controversy is related to risk:benefit considerations. More specifically, a positive result can lead to invasive procedures that cause significant morbidity, psychological stress, and significant economic cost. Some health care professionals believe that the rate of false positives for these tests (PSA and mammography) is unacceptably high, on balance causing more harm to people that do not have the disease than benefit to people who do. At this time, this controversy is far from resolution and will only be resolved by continued and diligent analysis of test performance and outcomes. Another issue raised is that, while for some cancers early detection can be life-saving, for others there is no evidence that finding the cancer early allows any intervention that improves outcome in terms of quality or quantity of life. In these cases, early detection may just create unnecessary and unproductive anxiety that reduces quality of life for some individuals.

This raises an important concern for the pet owner: if cancer tests approved by the FDA and performed under CLIA standards are subject to question and controversy, how do we determine if and when it is reasonable to do a test offered to detect, diagnose, or prognosticate cancer in pets? As explained in more detail below, the answer is not easy, and it requires vigilance, advocacy, and awareness by both pet owners and veterinary care team members.

Even beyond the potential reliability, the greater consideration should be, "Would early detection allow me to intervene and improve the outcome for my pet?" In the case of human patients, for example, a high PSA

test result would lead the urologist to do additional tests focused on the possibility that the patient has prostatic disease (imaging studies, biopsy, etc.). The subsequent identification of prostate cancer in that patient would lead to potentially lifesaving, or life-extending therapy, again, focused on the prostate. The same applies to tests which measure biomarkers that are highly specific for cancers of one type or in a single location. On the other hand, tests for early detection that are not highly specific – that is, they are associated with processes such as cell division that are common to many cancers, and they have no relation to an anatomic determinant (organ or tissue specificity) – may not be especially useful.

Consider a test that can detect any cancer, or a specific cancer that can occur in a variety of organs, like canine hemangiosarcoma. If the test is sufficiently sensitive to detect the cancer before it is visible by advanced imaging studies or other methods, the veterinarian and the owner are left at a loss for what to do. Should they use cytotoxic therapies on a patient that is not ill, risking possible severe side effects? Would the tumor cells at that stage be sensitive to such therapies? Should they remove organs where the incipient tumor may be present, and what complications might arise? Is there a guarantee that the tumor is not present elsewhere? How does the veterinarian counsel the owner who may now be helpless to help their pet? Is the stress of knowing that a pet might develop cancer and die justifiable when an intervention does not exist? In this scenario, testing may be not only of little use, but it also may be ethically questionable.

The diagnostic process

There is neither a single rule nor a single path to reach a cancer diagnosis. Some tumors are quite obvious, manifesting as obvious lumps or bumps outside the body, or easily palpated masses inside a body cavity. Other tumors are quite difficult to detect, as they may be nestled deep in an organ, inside the brain, or distributed throughout the body with no external indication of their existence.

Some patients can have severe clinical signs associated with the mass, such as pain, ulceration, discoloration, etc. Others can have severe, but non-specific signs of illness, such as lethargy, inappetence, diarrhea, etc. And others still, can be insidious, causing no signs until they are so advanced as to be untreatable.

This, along with the relatively high prevalence of cancer (especially in older animals) means veterinarians must maintain “cancer” in their list of “rule outs” for any ill dog until proven otherwise. Then again, how can it be proven that an animal does not have cancer? For the answer to that question, read on.

Routine laboratory testing

Current veterinary medical standards promote the use of minimal databases that allow the veterinary professional to monitor his or her patients’ health using a collection of biomarkers. These biomarkers include information obtained from complete blood counts, serum and urine chemistry profiles, and diagnostic imaging studies. In addition to other routine components of the physical exam, these tests allow the veterinary professional to ascertain changes that are inconsistent with those expected from normal ageing.

When cancer is suspected, for example, if there is an abnormal growth outside the body or within a body cavity, additional tests are done to “rule in” or “rule out” the various pathological processes that can explain the clinical signs. Among the common rule-outs in a cancer work-up, one must consider that lumps and bumps can be caused by inflammation; anemia (low red blood cell counts) can be caused by renal failure; and, masses in the spleen can be hematomas. It is difficult to prove a negative, so while positive results can provide a definitive diagnosis, negative results might only change the relative order of potential diagnoses. It is a common result that one test leads to other tests in the diagnostic process. Yet, it is essential to understand that ordering tests prematurely is not always efficient, and it can derail the diagnostic process. This is because a specialized test interpreted outside its proper context can lead the diagnostician astray, masking both the real diagnosis and the most useful tests that should be performed.

Recent and specialized laboratory tests for cancer: Do's and Don'ts.

The severity of cancer has led to multiple areas of testing and development. This section is focused on just a few assays that were developed in research laboratories and subsequently commercialized. The focus is purposeful, as these are the tests for which we receive many inquiries, but it is not meant to detract from the utility of other tests that veterinarians commonly use for cancer diagnosis.

Clonality testing for lymphoma: PCR for Antigen Receptor Rearrangements (PARR)

This test is based on detecting a signature that can distinguish whether a lymphocyte population arises from a single cell (monoclonal) or from many cells (polyclonal). Every lymphocyte in the body acquires a unique "antigen receptor" through a mechanism that involves moving segments of DNA (rearrangements) to help prepare these cells to protect the body from the many different infectious agents that can be encountered over a lifetime. The diversity of the lymphocyte pool, and therefore the precision of the clonal signature, increases through a process called somatic mutation that takes place in activated lymphocytes.

Antigen receptor rearrangement is a normal process; the test utilizes PCR to amplify sequences from all the lymphocytes present in a sample. Essentially, the main result is to say whether all or most of the sequences are the same, which means the population is clonal (and therefore more likely to originate from a cancerous process), or if there are many different ones, which means the population is polyclonal (and more likely to occur in response to an infectious agent or an allergen). Lymphocytes undergo clonal expansion when they are activated. Under conditions where normal lymphocytes are activated, a few or many clones undergo this expansion and create a polyclonal response. When malignant lymphocytes divide, the hallmark is the presence of a single clonal population.

The test is offered by a number of different laboratories, each of which has established rigorous standards for quality control. The estimated sensitivity of the test is 75%, and the specificity is about 94%. Conditions that might produce false positive or false negative results have been thoroughly evaluated and published. This is a powerful test, but is not meant as a stand-alone tool. Rather, it is meant as an adjunct test that adds a level of information or confirmation to other commonly used diagnostic tests. Nevertheless, after more than 10 years of refinement, this test is now considered among the core diagnostic tools for lymphoma.

P-glycoprotein mutation

This test is meant to identify dogs that carry a mutation in the ABCB1 gene, which encodes a membrane transport protein called P-glycoprotein (PgP) or multi-drug resistance (MDR) protein. ABCB1 is a member of a large family of genes that encode specialized proteins that pump nutrients into and toxins out of cells. This protein was originally discovered as a culprit for chemoresistance in cancer: it is commonly upregulated in tumors where it serves to pump drugs out of cancer cells before they can cause the intended damage. However, it also serves a protective function by preventing drugs from crossing into organs where they can cause irreparable damage.

When daily heartworm preventatives became widespread in the early 1990s, there was a curious association with neurologic toxicity in collies and other herding dogs that received these drugs. This led to the discovery of a mutation in the ABCB1 gene that is present in as many as 10-12% of dogs. The mutation encodes a defective, inactive product, and the prevalence is highest in herding breeds. The mutation does not seem to carry ill effects other than the high risk for toxicity with drug treatment. Testing is indicated, especially for dogs from high-risk breeds (or dogs that have these breeds in their ancestry), as an aid to plan heartworm prevention strategies or in the case of dogs with cancer, to develop individualized chemotherapy protocols. It is available through the Washington State Clinical Pharmacology lab.

OncoPet RECAF test

Recently, OncoPet Diagnostics and BioCurex announced commercialization of a blood test for cancer detection in companion animals. This test is based on detection of a protein in the blood that is, or is similar to the alpha-fetoprotein receptor. Alpha-fetoprotein is involved in the regulation of growth and immune function. The receptor for alpha-fetoprotein is incompletely characterized; the reagent used for this test was made against a membrane extract of human breast cancer cells and recognizes one of several proteins that bind alpha-fetoprotein.

This same test has been commercialized for human patients with cancer, but as of late 2011 it had yet to receive FDA approval, although assessment was in progress. The reagent recognizes a protein in dog cells, and so it is reasonable to hypothesize that this protein might also be increased in dogs with cancer. However, there is considerable controversy even among the experts in the field regarding the potential of alpha-fetoprotein receptors to act as "universal tumor markers." Data regarding the presence of this protein in dogs with cancer are only available on the company's website and have not yet been published in a peer reviewed journal, so scientists outside the company have not thoroughly evaluated this test. The company's own question/answer section and the disclaimers in their data sheet indicate that there is not yet enough information to decide under what conditions this test will be useful as a diagnostic tool for cancer in pets. While running the test is not harmful, an incorrect diagnosis can have profound consequences, and so this test should be used judiciously and always in combination with other established diagnostic tests.

VDxI-TK test for cancer

Thymidine kinase (TK) is an enzyme expressed in cells that are undergoing division. TK is released into the blood and serves as a biomarker for cell proliferation. Thus, TK levels are higher in any condition when there are rapidly dividing cells. This can include non-malignant conditions like pregnancy, growth, and inflammation, as well as malignant cancers. Several publications support an association between elevated TK levels in the blood and cancer. An advantage of this test, like RECAF, is that it is minimally invasive, requiring only a blood sample. In the case of hemangiosarcoma, the reported sensitivity is 50%. This means the false negative rate is quite high (it will have negative calls in half of the cases where dogs have a tumor). This may be due to confounding by other non-malignant conditions (see above). They reported specificity is 90%, but the test cannot distinguish among different types of cancer so this specificity can only be achieved when the TK test is combined with other diagnostic tests. Moreover, its predictive value is not known.

Other tests perform equivalently or better that the TK test, but few can be done in a blood sample. It is fair to say that there also is not enough information to decide when this test should be applied. It is apparent that when used, the test should be combined with a diagnostic biopsy that can establish the cancer type, and with imaging studies to stage the tumor. Perhaps the VDxI-TK test is better suited to monitor responses to therapy, for example in dogs with cancer where the basal TK activity was known, and where it diminished when the dog went into remission. In such cases, a persistent elevation back towards the baseline could indicate relapse.

VDxI canine-specific C-reactive protein test and INCaSe

C-reactive protein (CRP) is an "acute phase protein" produced by the liver as an early component of systemic inflammation (involving the whole body and not just a small local area). The white blood cells that are responsible for inflammation produce little or no CRP. Instead, they release other factors (called interleukins) that instruct the liver to produce and release CRP. Among other functions, CRP coats and inactivates bacteria, which helps the body to eliminate infection.

Inflammation is a basic response to many abnormal states, and so while the CRP test is relatively specific to detect the presence of systemic inflammation, it does not necessarily provide information about the

cause of inflammation. Elevated CRP has been documented in dogs with active bacterial or fungal infections, in dogs with some viral infections, in dogs with certain chronic inflammatory diseases such as some types of arthritis, and occasionally, in dogs with cancer. The VDxI CRP point of care test (TECO) has high sensitivity (94-96% in blood or serum) and specificity (83-91% in blood or serum). So the test is quite useful to detect or monitor inflammation. As noted above, however, the test cannot discriminate among the many different conditions that cause inflammation.

It has been proposed that the combination of VDxI-TX and VDxI-CRP, sold under the name INCaSe, can be used as a method to screen otherwise healthy pets for cancer, in other words, as an early detection test. The principle of combining these two tests is sound, in that it might help to establish the presence of inflammation and confirm or eliminate one of the common causes that confound interpretation of the TK test. However, this test will fail to perform in cases where cancer and inflammation co-exist and both are positive (as noted above, positive CRP tests have been documented in dogs with neoplasia), and in cases where the TK falls below the reference or is negative, such as 50% of hemangiosarcomas. As is the case for other tests described here, there is no peer-reviewed information that scientists outside the company can use to thoroughly evaluate this test, and so until robust data are available to confirm its utility, the INCaSe test should not be considered as a standard to screen otherwise healthy pets for cancer.

PetScreen Lymphoma Blood Test (LBT)

The principle of this test is based on the concept that cancer cells (lymphoma cells in this case) make proteins that are different from those made by normal cells, and that these proteins can be detected in blood or serum using very sensitive methods. Because the identity of the proteins is unknown, PetScreen developers sought to find those proteins using methods that allow for comparison of all, or almost all the proteins that can be measured in a serum sample. This method, called “proteomics” has been used in the research setting for more than a decade and it holds promise in the diagnostic arena.

Results have been published from one study evaluating the PetScreen LBT. In this study, there was a profile of proteins that could distinguish if serum samples originated from dogs with lymphoma or from dogs without lymphoma with 91% specificity and 75% sensitivity. The PetScreen scientists that conducted this study estimated a positive predictive value of 80% and a negative predictive value of 88%. They further examined samples from dogs that had no lymphoma at the time of testing to see how many would develop lymphoma 3 – 6 months later. Of the 96 dogs in this group, 30 had a positive test result, and of these, 24 had been diagnosed with lymphoma at the time of follow up.

On the surface, these results would appear remarkable, and they do indeed represent a large step towards advanced diagnostics. But there are a number of problems that would preclude us from recommending this test as a routine screening or diagnostic test. First, the company has not disclosed the nature of the proteins that allow them to distinguish between “normal” and “lymphoma”, so it is impossible for any independent observer to establish a cause-effect relationship. The published data refer to two proteins (one large and one small) that are the principal determinants, and 8 surrogates that can be used when the results from the first two are equivocal. The lack of a cause-effect relationship is not a problem for some biomarkers that perform admirable in the diagnostic realm (for example, CRP is a very reliable marker of inflammation that does not distinguish among the many causes of inflammation). But in the case where the information is absolutely means that the interpretation of the results must rely partly on faith – an approach that runs counter to evidence based medicine.

Another problem with LBT test is that the population used for validation had some level of uncertainty. Some dogs were diagnosed based on “clinical judgment”, a measure that is subjective. In addition, we recognize that “lymphoma” is a general descriptor for a variety of diseases that arise from different cells and have different behavior and response to therapy. In this study, the investigators did not distinguish

among lymphoma subtypes, so it is unclear if the test is useful for only one or a few common types of lymphoma, or if it is useful for any type of lymphoma.

As is true for other tests described here, it is important to understand when and where this test is likely to provide benefit. For example, in the case of a dog that has large lymph nodes and where a biopsy is obtained, the LBT test would be redundant (would not add any significant information). To date, its value to monitor recurrence is unknown, but it would be justifiable to use the test experimentally to assess this, as it might be able to detect relapse before other methods. As far as "screening," this test is unlikely to have benefit when used in a dog where the probability of lymphoma is remote. The reported predictive value did not take into account the likelihood of disease, and the test has yet to be rigorously evaluated in dogs with a variety of other conditions. The company reports that "immune and inflammatory conditions" did not interfere with performance, but they do not report if other tumors might. Finally, the use of the LBT in an otherwise healthy dog where the probability of lymphoma is greater must be balanced with the value that such information would provide. In other words, what would be the next steps to confirm the diagnosis and how would it change the treatment, if at all? These are questions that every veterinarian should apply to every test. When a test result does not substantially advance the diagnosis or help develop a treatment plan, it can still be justified if there is no downside (negative outcome). But when a test result can raise the index of suspicion for a terminal disease without providing guidance for management, the significant downside must be considered before the test is adopted for routine use.

Zen – a practical approach to cancer

The Wikipedia description of Zen is "a practice that emphasizes the personal expression of experiential wisdom in the attainment of enlightenment." In the area of cancer diagnostics, this Zen can be defined as the wisdom and enlightenment to be rational and use only those tests that are likely to improve the diagnostic accuracy, inform prognosis, or guide therapy. Owners and veterinarians should communicate clearly, carefully, and thoroughly. For every test there must be a reason and a purpose. Screening tests that do not meet these goals probably have little value in the diagnostic process for the cancer patient.

The lack of regulation for veterinary laboratories means that owners and veterinarians must be even more vigilant. The potential severity of cancer creates a sense of urgency to develop a management plan. It is then when people are most vulnerable, and when it will be most important to avoid substituting reason for emotion. The promise of discovery is great, and the introduction of the tests described here, as well as many others simply illustrates the magnitude of the problem and its societal importance. In the end, only those tests that prove to be truly useful will survive the test of time. But until then, it is practical to heed the old adage "buyer beware."

IMPORTANT NOTE from Rochelle Lesser, Land of PureGold Foundation Founder, May 2012

This article represents the opinion of two people, pivotally involved in cancer research and companion animal diagnostics. One can never take for granted, how much respect we have for these particular authors' work and accomplishments.

The fact that Dr. Modiano elicited input from a fellow researcher, speaks volumes about his commitment and collaborative nature. We are very appreciative of such thoughtful guidance.