Evaluation of a bladder tumor antigen test as a screening test for transitional cell carcinoma of the lower urinary tract in dogs

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Objective—To evaluate the veterinary version of the bladder tumor antigen (V-BTA) test as a screening test for transitional cell carcinoma (TCC) of the lower urinary tract of dogs.

Animals—229 client-owned dogs.

Procedure—Urine samples from dogs were shipped overnight to a single laboratory to facilitate testing within 48 hours of collection by use of the V-BTA rapid latex agglutination urine dipstick test. Groups of dogs included the following: 1) dogs with TCC of the lower urinary tract, 2) healthy control dogs, 3) unhealthy control dogs with non-TCC urinary tract disease, and 4) unhealthy control dogs without urinary tract disease. Test sensitivity and specificity were calculated by use of standard methods. Logistic models were developed to assess the effect of disease status, test conditions, urine composition, and signalment on the performance of the V-BTA test.

Results—A total of 229 urine samples were analyzed, including 48 from dogs with suspected (n = 3) or confirmed (45) TCC. Test sensitivities were 88, 87, and 85% for all dogs with (suspected and confirmed) TCC, dogs with confirmed TCC at any site, and dogs with confirmed TCC of the urinary bladder, respectively. Test specificities were 84, 41, and 86% for healthy control dogs, unhealthy control dogs with non-TCC urinary tract disease, and unhealthy control dogs without urinary tract disease, respectively. The test performed slightly better on centrifuged urine samples than on uncentrifuged urine samples.

Conclusions and Clinical Relevance—Our results indicate that the V-BTA test is useful in screening for urinary tract TCC in dogs. (*Am J Vet Res* 2003;64:1017–1020)

Primary urinary bladder malignancy in dogs comprises 0.5 to 1% of all canine cancers in published reports.[1-3] However, to our knowledge, no large reviews of the prevalence of urinary bladder cancer in dogs have been reported within the past decade. Of the malignancies known to occur in the urinary bladder of dogs, **transitional cell carcinoma (TCC)** is the most common, accounting for approximately a half to three fourths of all reported instances.[4-6] Successful treatment of this malignancy in dogs is often precluded by the invasive nature of the disease at the time of diagnosis and the high metastatic rate, ranging from 14 to 37%.[1,7,8] Earlier detection may enhance the treatment outcome for dogs affected with TCC.

The **veterinary version of the bladder tumor antigen (V-BTA)** test[a] is a rapid latex agglutination dipstick test that has been developed to permit the qualitative detection of tumor analytes in urine as an adjunct in the early diagnosis of TCC in dogs. The test, easily performed in the veterinarian's office, uses antibodies to a urinary bladder tumor-associated glycoprotein complex detectable in the urine. The analytes detected by the V-BTA test have been isolated and characterized from the urine of humans with urinary bladder cancer and shown to contain high molecular weight (16 to 165 kd) glycoproteins, which appear to consist of complexes of basement membrane proteins and, in some instances, may also contain immunoglobulin. Invasive urinary bladder tumors possess the ability to degrade the basement membrane into fragments of its basic components (eg, type-IV collagen, fibronectin, laminin, and proteoglycans).[9-11] The loss of basal lamina proteins leads to the formation of detectable protein complexes in urine. These components are released into the urine where they combine to form basement membrane complexes. The V-BTA test requires 0.5 mL of test urine. The urine sample is buffered and centrifuged, then mixed with latex particles coated with human IgG and blocking agents. If the urinary bladder tumor analytes are present in the urine at a substantial concentration, they will combine with the latex particles to produce an agglutination reaction. Following the formation of agglutinates, a visual color change differentiates positive from negative results by use of a specially prepared strip. The purpose of this study was to evaluate

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the V-BTA as a screening test for TCC of the lower urinary tract in a large population of dogs.

Materials and Methods

Urine samples were collected at 4 veterinary medical teaching hospitals between June 1999 and November 2001. Urine samples were shipped overnight to a single laboratory[b] to facilitate testing within 48 hours of collection. Individuals performing the test were blinded with regard to patient diagnosis.

Groups of dogs included the following: 1) dogs with TCC of the lower urinary tract, 2) healthy control dogs, 3) unhealthy control dogs with non-TCC urinary tract disease, and 4) unhealthy control dogs without urinary tract disease. A diagnosis of TCC was categorized as suspected when clinical signs and imaging suggested a mass and as confirmed when cytologic or histologic evaluation was consistent with a diagnosis of TCC. For the purpose of analysis, dogs with prostatic tumors were categorized as having TCC.

Test sensitivity and specificity were calculated by use of standard epidemiologic methods. Additionally, forward stepping logistic regression was used to develop models that determined the effect of independent variables on the probability of a positive V-BTA test result. In this analysis, the data set included test results (n = 368) for urine samples that were processed in duplicate prior to and following centrifugation (184). Independent variables considered in this analysis included patients sex, age, method of urine collection, presence of a TCC, presence of a urinary tract infection, presence of a urinary tract disease other than neoplasia or infection, presence of other nonurinary tract illness, urine specific gravity (< 1.008, 1.008 to 1.012, or > 1.012), aciduria (pH, < 6.0), alkaluria (pH, > 7), and the degrees of pyuria (0, < 5, 5 to 100, or > 100 WBCs/**high-power field** [**hpf**; 400× magnification]), proteinuria (none, trace, 1 to < 50, 50 to < 250, or ≥ 250 mg/dL), glucosuria (0, trace to < 50, 50 to < 250, or ≥ 250 mg/dL), ketonuria (0, trace to < 250, or ≥ 250 mg/dL), bilirubinuria (present or absent), and hematuria (0, < 5, 5 to < 20 [1+], 20 to < 100 [2+], or ≥ 100 RBCs/hpf [3+]). At each step, the variable with the smallest *P* value to enter was added to the model until no variable had a *P* value to enter of < 0.05. **Odds ratios (ORs)** were calculated for all variables significantly ($P < 0.05$) associated with the probability of a positive V-BTA test result.

Results

A total of 229 urine samples were analyzed, including 48 from dogs with suspected (n = 3) or confirmed (45) TCC of the lower urinary tract. Urine samples were obtained from 31 dogs with TCC confined to the urinary bladder; 13 with TCC of nonurinary bladder sites including kidney (n = 1), urethra (5), or prostate (7); and 4 with involvement of the urinary bladder and another urinary site. Of the 45 dogs with confirmed TCC, confirmation was by cytologic evaluation (n = 32), histologic evaluation (9), or both (4). The control groups consisted of 82 healthy control dogs, 71 unhealthy control dogs with non-TCC urinary tract disease, and 28 unhealthy control dogs without urinary tract disease.

Test sensitivities were 88, 87, and 85% for all dogs with (suspected and confirmed) TCC, dogs with confirmed TCC at any site, and dogs with confirmed TCC of the urinary bladder, respectively. Test specificities were 84, 41, and 86% for healthy control dogs, unhealthy control dogs with non-TCC urinary tract disease, and unhealthy control dogs without urinary tract disease, respectively. When performed on centrifuged samples, as per the label directions, the test performed slightly better than on uncentrifuged samples (**Table 1 and 2**). For all groups other than the unhealthy control dogs without urinary tract disease, test sensitivity (for dogs with TCC) and specificity (for dogs without TCC) were improved by centrifugation of urine prior to testing over that achieved with uncentrifuged urine samples. Independent variables significantly associated with a positive V-BTA test result included advanced age (OR, 0.44), presence of a TCC (OR, 6.29), presence of a urinary tract infection (OR, 3.26), aciduria (pH, < 6.0; OR, 4.95), glucosuria (OR, 3.15), proteinuria (OR, 2.99), 2+ hematuria (OR, 5.08), and 3+ hematuria (OR, 71.43). All remaining factors were not significantly associated with test results.

Table 1—Comparison of the veterinary version of the bladder tumor antigen (V-BTA) test sensitivity for centrifuged versus uncentrifuged urine samples from dogs with transitional cell carcinoma (TCC)

		Sensitivity (%)	
TCC category	**No. of dogs**	**Centrifuged**	**Uncentrifuged**
Confirmed (any site)	45	85.7	83.3
Confirmed (urinary bladder)	31	84.4	81.5
Confirmed and suspected	48	86.7	85.0

Table 2—Comparison of the V-BTA test specificity for centrifuged versus uncentrifuged urine samples from control dogs with no known TCC

		Specificity (%)	
Control category	**No. of dogs**	**Centrifuged**	**Uncentrifuged**
Healthy	82	87.6	82.2
Unhealthy with UTD*	71	45.5	40.8
Unhealthy without UTD	28	84.2	88.0

*Unhealthy control dogs with non-TCC urinary tract disease. UTD = Urinary tract disease.

Discussion

In 2 previous studies,[12,13] the use of a bladder tumor antigen test had been evaluated by comparing test outcome in dogs with confirmed (via pathologic findings on histologic or cytologic evaluations) lower urinary tract neoplasia with dogs without urinary neoplasia. The first report,[12] evaluating a first-generation bladder tumor antigen test, included 20 dogs with TCC, 19 healthy control dogs, and 26 control dogs with non-neoplastic urologic disease. Comparisons among groups indicated an overall test sensitivity (likelihood of detecting TCC in affected dogs) of 90% and specificity (likelihood of a negative test result in a dog that is free of TCC) of 78%. False-positive results were found for dogs with glucosuria, proteinuria, pyuria, and hematuria.[12] The test, now known as the V-BTA test, was recently evaluated on urine samples from 20 dogs with lower urinary tract neoplasia, 18 dogs without urinary tract disease, and 16 dogs with nonmalignant urinary tract disease.[13] Urinary tract neopla-

sia included TCC, prostatic adenocarcinoma, and lymphoma. Test sensitivity was 90%, and specificity was higher than previously reported at 94.4%.[13] Our current study is unique in that it included unhealthy control dogs without urinary tract disease as an additional control group, and test outcome was compared between centrifuged versus uncentrifuged urine samples. The larger number of urine samples analyzed was also considered important for validating previously reported calculations of test sensitivity and specificity.

Our results indicate that centrifugation of urine samples is important for optimizing test performance. Test sensitivity and specificity were improved when urine samples were centrifuged prior to testing. This step is recommended in the current manufacturer's instructions yet was not routinely done in a previous study[13] of test performance.

The large number of urine samples tested in our study was sought in an effort to validate previous reports[12,13] of test performance. Previous reports[12,13] have included 65 or fewer dogs, with a total of 40 dogs with neoplasia. In our study, we tested 229 urine samples, including 48 from dogs with known (n = 45) or suspected (3) TCC. Our results indicate a test sensitivity of 88%, similar to the 90% sensitivity reported previously.[12,13] Test specificity in our study fell between the previously reported 78 to 94.4%,[12,13] with the specificity for control dogs, other than unhealthy control dogs with non-TCC urinary tract disease, ranging from 84 to 86%. As reported by Billet et al,[13] the test did not perform as well on urine samples from dogs with non-neoplastic urinary tract disease, in which false-positive results were found for some dogs with proteinuria or moderate to marked hematuria. However, the role of the V-BTA as a screening test (as opposed to a confirmatory diagnostic test) is validated by the high test sensitivity reported consistently in our study and in 2 previous studies.[12,13]

When examining the performance of the V-BTA test, the common concern has been that test performance is inadequate for routine diagnostic use. The focus has often been on the causes of false-positive test results, rather than on the merit of the test for screening and eliminating costly diagnostic procedures in dogs that are at low risk on the basis of V-BTA results. The low specificity of test results in our study for unhealthy control dogs with non-TCC urinary tract disease (41%) indicates that one should not indiscriminately use the test in all dogs with clinical signs of urinary tract disease. These limitations have been discussed in detail in previous reports[12,13] that also indicated problems with false-positive test results associated with the V-BTA. Although centrifugation of urine samples prior to testing improved test performance, the issue of false-positive results remains.

Transitional cell carcinoma, comprising 0.5 to 1% of all neoplastic diseases in dogs, is a malignancy with which most practitioners are familiar. As approximately 50% of dogs > 10 years of age will die of cancer, the need and public demand for appropriate screening tests in veterinary medicine are apparent.[14,15] To better understand how the V-BTA test would perform in mass screening of apparently healthy dogs, population statistics should be taken into consideration. Considering that a population of aged dogs will experience approximately a 0.5% prevalence of TCC, it is possible to calculate the **positive predictive value (PPV)** and the **negative predictive value (NPV)** by use of the calculated sensitivity (88%) and specificity (84%) in our study. The PPV would be 0.028, and the NPV would be 0.999. Therefore, < 3% of dogs with positive V-BTA test results would be expected to have TCC. Under these circumstances, the use of the V-BTA test provides little diagnostic use, but would effectively eliminate concerns regarding TCC in dogs with negative test results, 99.9% of which would be free of this cancer.

If the test application were limited to geriatric dogs of a breed considered at high risk for TCC that also had stranguria and hematuria, the a priori probability of TCC increases. By restricting application of the V-BTA test to this highly targeted population, the de facto prevalence of TCC is increased to some value that is much higher than that in the previous example. For purposes of this illustration, the prevalence in this prescreened population is assumed to be 20%. Under these circumstances, the PPV increases to 0.272 (27% of dogs with a positive test result would have TCC), and the NPV would be 0.932. Although the PPV indicates that these test results will not provide a definitive diagnosis, a negative test result again provides a fairly confident rule-out (93%) of TCC, which is even more important in this high-risk group that is likely to be considered for additional expensive and invasive diagnostic procedures. Although the use of cancer-screening tests in veterinary medicine is uncommon, cancer-screening tests with a similar PPV for humans, such as the prostate-specific antigen measurement for men > 50 years of age,[16] are used routinely. With the prostate-specific measurement, a positive test result in a high-risk patient does not confirm a diagnosis, but indicates that additional diagnostic procedures are warranted. Conversely, a negative test result may permit clinicians to avoid expensive and invasive diagnostic procedures requiring sedation or anesthesia in patients with a low likelihood of cancer. Similar diagnostic planning may be provided by use of the V-BTA test in dogs at risk for TCC of the urinary bladder. By performing appropriate diagnostic testing on dogs with positive test results, the ultimate goal is to facilitate earlier detection and intervention that will improve responses to treatment for this highly fatal disease of dogs.

An additional potential benefit of the V-BTA test beyond diagnosis and early intervention is the impact this assay may have on the management of affected dogs. Not only will dogs with positive test results be targeted for more intensive diagnostic protocols, the manner in which these diagnostics proceed may be altered. For example, the use of cystocentesis in dogs with a positive V-BTA result might be considered contraindicated as a result of the increased risk of tumor seeding along the needle tract.[17]

The ultimate objective of any cancer-screening test is to permit earlier disease detection and subsequent reduction in cancer-related morbidity and death. As survival of dogs with TCC is strongly associated with tumor stage at the time of diagnosis, it is reasonable to

hypothesize that early detection of TCC may lead to improvement in outcome.[7,18] Although not tested in our group of dogs, urine-screening tests may have clinical use as a method to monitor response to treatment for TCC and detect tumor recurrence at the primary site.

[a]VBTA Test, Alidex Inc (subsidiary of Polymedco Inc), Redmond, Wash.

[b]Alidex Inc, (subsidiary of Polymedco Inc), Redmond, Wash.

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