Evaluation of a urine dipstick test for confirmation or exclusion of proteinuria in dogs

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Objective—To evaluate a urine dipstick test as a possible replacement for urine protein-to-creatinine (UPC) ratio for identifying proteinuria in dogs.

Sample Population—507 urine samples from adult dogs.

Procedures—Urine dipstick, UPC ratio, specific gravity (USG), and sediment testing were performed on 507 samples. With UPC ratio as the reference criterion, diagnostic accuracy of the urine dipstick test was calculated for the entire data set and for urine samples grouped by USG (≤ 1.012 or > 1.012; < 1.030 or ≥ 1.030). A UPC ratio < 0.2 was used to indicate absence of proteinuria.

Results—The sensitivity of the urine dipstick test for detection of proteinuria was > 90% when 0 mg of protein/dL (a 0+ result) was used to indicate a negative test result, and the specificity ranged from 40% to 60%, depending on the USG. Sensitivity decreased to a range of 56% to 81% when 30 mg of protein/dL (a 1+ result) was used as the cutoff, depending on the USG, but the specificity increased to > 90%. The likelihood of correctly identifying nonproteinuric dogs was low when the USG was ≤ 1.012, particularly when samples with a 1+ result were considered negative.

Conclusions and Clinical Relevance—For dogs with a dipstick-test result of 1+ and USG ≤ 1.012, proteinuria should be assessed by use of the UPC ratio; dogs with a USG value > 1.012 are likely nonproteinuric. When used together, the urine dipstick test and USG measurement were reliable as a rapid alternative to UPC ratio determination in dogs in this study. (Am J Vet Res 2010;71:235–240)

Results of several studies1–5 have suggested the importance of correctly identifying and treating proteinuria in dogs, as the degree of urine protein loss is highly associated with morbidity and death attributable to uremic crises. In contrast to the situation in humans, daily urinary excretion of proteins cannot be routinely measured in dogs, and the most widely used method for detection of proteinuria is the UPC ratio.5–8 The UPC ratio correlates well with daily protein excretion and its use is practical because the value can be assessed by use of 1 urine sample.5–8 Veterinary practitioners usually send samples for UPC ratio measurement to external laboratories after proteinuria has been identified with the urine dipstick test, which is the most accepted of the so-called patient-side tests and is easy to use as well as inexpensive. The usefulness of the dipstick test to screen for proteinuria, particularly to detect albuminuria, has been reported.5,8–10

In clinical practice, however, once urine has been analyzed with a dipstick test, practitioners would undoubtedly benefit from knowing whether the sample should be tested further to determine the UPC ratio, which precisely quantifies protein loss. Because it is generally accepted that a urine dipstick test can yield false-negative results, possibly leading to diagnostic errors and, in turn, to incorrect therapeutic decisions in some dogs, many veterinarians prefer to directly determine the UPC ratio in all patients. This approach...
may increase costs and delay definitive exclusion or confirmation of proteinuria. If the urine dipstick test could identify dogs with and without proteinuria, assessment of the clinical status of dogs would become more straightforward, with obvious advantages for dogs, owners, and veterinarians.

The purpose of the study reported here was to assess whether the urine dipstick test correctly identified nonproteinuric and proteinuric dogs, with UPC ratio used as the reference criterion and cutoffs of the IRIS classification system\textsuperscript{11} used to indicate urine protein status. Because urine concentration may influence dipstick readings (eg, low specific gravity may obscure proteinuria), concordance between dipstick results and the UPC ratio was also assessed taking into account the effect of USG.

**Materials and Methods**

**Animals**—Records of 1,978 consecutive adult dogs evaluated at the Clinica Veterinaria Pirani of Reggio Emilia (Italy) and the Veterinary Teaching Hospital, Universitat Autònoma de Barcelona (Spain), between January 2005 and September 2008 were retrospectively reviewed. Dogs were included in the study when they had undergone a complete urinalysis, including dipstick test, UPC ratio determination, and USG measurement, and any urine sediment was inactive. In addition, urine samples that were abnormally colored or turbid or had a pH > 7.5 were excluded. Urine samples were collected as part of the minimal database required for dogs admitted with various clinical signs suggestive of renal, urological, endocrine, and metabolic disorders. In all dogs, urine samples were collected by cystocentesis.

At the authors’ institutions, urine samples are routinely examined within 4 hours after collection or are stored at 4°C and analyzed within 24 hours after collection. The sediment is obtained by centrifugation (10 minutes at 900 × g) of 5 mL of urine, followed by removal of 4.5 mL of supernatant. The supernatant is used to determine USG and detect proteinuria and is then microscopically examined after resuspension in the remaining 0.5 mL of urine. The USG is measured with a hand refractometer\textsuperscript{9} that is calibrated daily with distilled water. Proteinuria is assessed with a urine dipstick test\textsuperscript{9} as described by the manufacturer. In brief, the measuring stick is immersed in the supernatant and a reading is obtained 60 seconds later, after removal of excess urine. Readings of the colorimetric scale are expressed as follows: 0+ = negative (0 mg/dL), 1+ = 30 mg/dL, 2+ = 100 mg/dL, and 3+ = 500 mg/dL.

To calculate the UPC ratio, urine protein concentration is measured by use of pyrogallol red\textsuperscript{1} and creatinine concentration is measured in undiluted urine with the Jaffé method.\textsuperscript{4} Analyte concentrations are measured in an automated spectrophotometer.\textsuperscript{7} The degree of proteinuria assessed with the UPC ratio is classified in accordance with the scheme proposed by the IRIS.\textsuperscript{11} Therefore, dogs with a UPC ratio < 0.2 are deemed nonproteinuric, dogs with a UPC ratio from 0.2 to 0.5 have borderline proteinuria, and dogs with a UPC ratio > 0.5 have frank proteinuria. For the purpose of the present study, data for dogs with borderline and frank proteinuria were grouped together because, in the clinical setting, dogs with either type of proteinuria should be evaluated further to characterize the underlying renal disease.

**Statistical analysis**—All statistical analyses were performed by use of a spreadsheet application,\textsuperscript{4} with a set of macroinstructions.\textsuperscript{6} The diagnostic agreement between urine dipstick-test results and UPC ratios was calculated by means of the Cohen $\kappa$ coefficient, which corrects the observed agreement for the probability that the agreement occurred by chance. Briefly, a $\kappa$ coefficient $\leq 0$ indicates that there is no agreement other than that which would be expected by chance, whereas a value of 1.00 indicates complete agreement. The strength of agreement was further classified as almost perfect for values from 0.81 to 1.00, substantial for values from 0.61 through 0.80, moderate for values from 0.41 through 0.60, fair for values from 0.21 through 0.40, and poor for values $\leq 0.20$.\textsuperscript{12}

Agreement between results of the 2 assessment methods was evaluated by considering urine samples with negative (0+ or 0 mg of protein/dL of urine) dipstick-test results as dipstick-negative and those with positive ($\geq 1+$ or $\geq 30$ mg of protein/dL) dipstick-test results as dipstick-positive. The analysis was then repeated by considering urine samples with negative dipstick-test results (0+) or with 30 mg of protein/dL (1+) as dipstick-negative and samples with $> 30$ mg of protein/dL ($\geq 2+$) as dipstick-positive. In the analysis of agreement, the UPC ratios were used to classify samples as negative (nonproteinuric) or positive (proteinuric).\textsuperscript{11} The aforementioned analyses were performed by use of the entire data set, irrespective of urine concentration and after grouping samples by USG ($< 1.030$ vs $\geq 1.030$ and $\leq 1.012$ vs $> 1.012$). A cutoff of 1.030 was used because it is believed to distinguish adequately concentrated urine from uncentrated urine in dogs.\textsuperscript{13-16} A cutoff of 1.012 was also used because it is generally accepted as the upper reference limit for isosthenuric dogs.\textsuperscript{14-16}

Urine protein-to-creatinine ratio was used as the reference criterion for detection of proteinuria, and the diagnostic performance of the urine dipstick test was assessed by counting the number of true-positive results (ie, dogs classified as proteinuric with both methods), true-negative results (ie, dogs classified as nonproteinuric with both methods), false-positive results (ie, dogs classified as proteinuric only with the urine dipstick test), and false-negative results (ie, dogs classified as proteinuric only with the UPC ratio but not the urine dipstick test). These data were used to calculate sensitivity, specificity, and negative and positive predictive values of the urine dipstick test.\textsuperscript{17} In addition, sensitivity and specificity values were used to calculate positive and negative likelihood ratios\textsuperscript{38} and to design an ROC curve to evaluate the discriminatory power of the urine dipstick test for assessment of proteinuria in dogs.\textsuperscript{19} As for assessment of intertest agreement, urine samples with negative (0+) dipstick-test results were considered dipstick-negative and those with positive ($\geq 1+$) dipstick-test results were considered dipstick-positive. The preceding calculations, including sensitivity, specificity, negative and positive predictive values, and likelihood ratios, were performed by use of the entire data set or
Results

Urine samples—Five hundred seven urine samples were included in the study. The mean USG value was 1.023 (range, 1.002 to 1.060). Among samples, 371 (73.2%) had a USG < 1.030, 136 (26.8%) had a USG ≥ 1.030, 140 (27.6%) had a USG ≤ 1.012, and 367 (72.4%) had a USG > 1.012. The urine dipstick test yielded a 0+ reading in 116 (22.9%); 1+ reading in 164 (32.3%); 2+ reading in 105 (20.7%); and 3+ reading in 122 (24.1%) samples. The mean UPC ratio was 2.16 (range, 0.00 to 37.44). Among samples, 191 (37.7%) had a UPC ratio > 0.2 and 316 (62.3%) had a UPC ratio ≥ 0.2.

Diagnostic agreement—Cohen κ values for agreement between urine dipstick-test results and UPC ratios as indicators of proteinuria were summarized (Table 1). When only the 0+ urine samples were considered dipstick-negative, the diagnostic agreement was moderate, whether the entire data set was used or samples were grouped by USG. When 0+ and 1+ samples were considered dipstick-negative, the diagnostic agreement increased, particularly at high USG values.

Discriminatory power of the dipstick test—The AUC obtained when the entire data set was included in the analysis was 0.89 (95% CI, 0.87 to 0.92; P < 0.001; Figure 1). When samples were grouped by USG, the AUCs remained similar to those obtained when the entire data set was included (Figure 2), although agreement was slightly better for samples with a higher USG (USG ≥ 1.030, AUC = 0.93 [95% CI, 0.89 to 0.97; P < 0.001]; USG > 1.012, AUC = 0.92 [95% CI, 0.90 to 0.95; P < 0.001]), compared with agreement for samples with a lower USG (USG < 1.030, AUC = 0.90 [95% CI, 0.87 to 0.93; P < 0.001]; USG ≤ 1.012, AUC = 0.86 [95% CI, 0.81 to 0.92; P < 0.001]).

Diagnostic accuracy—Results for diagnostic accuracy and predictive ability of the urine dipstick test for identifying proteinuria, with the UPC ratio as the reference criterion, were summarized (Tables 2 and 3). When data for samples analyzed irrespective of the USG and data for samples grouped by USG were used in calculations, the sensitivity of the urine dipstick test was >90% when only samples that were negative (0+) were considered dipstick-negative and all the other samples were included in the dipstick-positive group. When the same dipstick-test result classification system was used, the negative predictive value was >80% to 90% (with the exception of samples with a USG ≤ 1.012 or < 1.030) and the negative likelihood ratio was low, particularly for samples with a USG ≥ 1.030. By contrast, when the definition of dipstick-negative was expanded to include samples classified as 1+ and that of dipstick-positive included only samples classified as ≥ 2+, the sensitivity decreased but the specificity considerably increased, reaching values >95% in each instance. The positive predictive value and the positive likelihood ratio were also high. On the basis of the aforementioned results, a scheme for interpreting urine dipstick results according to USG was developed (Table 4).

Discussion

The present study revealed that the urine dipstick test has high discriminatory power for detection of proteinuria in dogs, particularly when USG cutoffs of >1.012 or ≥ 1.030 are used to designate proteinuria, as supported by the ROC curves. Similarly, at the same concentrations, diagnostic agreement of urine dipstick-test results with UPC ratios was substantial to almost
perfect when urine samples with 30 mg of protein/dL (1+) were classified as dipstick-negative (thus considering as dipstick-positive those samples with ≥ 100 mg of protein/dL [2+] ), whereas agreement was only fair when the USG was ≤ 1.012. By contrast, when the definition of dipstick-positive was expanded to include only the 0+ results, the agreement was moderate at most urine concentrations, except when USG was ≥ 1.030, in which situation agreement was only fair.

According to our suggested scheme for interpreting urine dipstick-test results, when the urine dipstick test yields a 0+ result, the negative predictive value of the test is high and the likelihood that the dog is nonproteinuric is also high as indicated by the low negative likelihood ratio (Table 4). In this instance, the veterinarian can exclude proteinuria in the associated dog according to the IRIS definition, particularly when the USG exceeds 1.030 (ie, the negative predictive value is 97.6% and the negative likelihood ratio is 0.04). For these dogs, we have concluded there is no need to send urine samples to external laboratories for UPC ratio determination. Likewise, when a urine dipstick test yielded results ≥ 100 mg/dL (2+), the positive predictive value of the test was always high in our study (93.0% to 99.4%) and the likelihood that a dog with this value is proteinuric is high, as supported by the high positive likelihood ratio obtained after exclusion of the data for 30 mg of protein/dL of urine (1+) from the dipstick-positive data. The veterinarian can be almost certain that a dog with a dipstick-test result of 2+ has a UPC ratio ≥ 0.2, regardless of the USG; urine samples from such dogs should be further evaluated to determine the UPC ratio. Exact quantification of the degree of proteinuria will clarify whether the dog has borderline or frank proteinuria, as defined by the IRIS classification system.11

Figure 2—Receiver-operating characteristic curves constructed for urine samples with a USG < 1.030 (A; n = 371), USG ≥ 1.030 (B; 136), USG ≤ 1.012 (C; 140), and USG > 1.012 (D; 367). See Figure 1 for remainder of key.
ent study were affected with a disease associated with the UPC ratio would be necessary. The positive likelihood ratio value of 2.3%, the probability would be high. When the estimate is corrected for the same dipstick-test results, the probability of proteinuria has swollen lymph nodes, anemia, and the when a dog living in an area in which leishmaniasis is endemic has (ie, 2.3%), would still remain low. On the other hand, after correction with the positive likelihood ratio value is most likely low. Thus, the probability that a 1+ dip sample obtained at that time may have a USG ≤ 1.012. The chance that the dog has a UPC ratio as the reference criterion for detection of proteinuria and a dipstick-test result considered negative when 0+ or positive when ≥ 1+.

Table 2—Diagnostic accuracy of the urine dipstick test as evaluated in 507 canine urine samples, with the UPC ratio as the reference criterion for detection of proteinuria and a dipstick-test result considered negative when 0+ or positive when ≥ 1+.

<table>
<thead>
<tr>
<th>Urine sample assessed</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>LR+</th>
<th>LR−</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
<td>94.3 (91.1–96.0)</td>
<td>50.8 (43.5–58.1)</td>
<td>1.92</td>
<td>0.11</td>
<td>76.0</td>
<td>84.3</td>
</tr>
<tr>
<td>USG &lt; 1.000</td>
<td>93.3 (90.4–96.0)</td>
<td>60.0 (49.4–89.9)</td>
<td>2.33</td>
<td>0.11</td>
<td>68.1</td>
<td>77.0</td>
</tr>
<tr>
<td>USG ≥ 1.030</td>
<td>98.4 (91.6–100.0)</td>
<td>41.7 (31.7–52.2)</td>
<td>1.69</td>
<td>0.04</td>
<td>52.9</td>
<td>97.6</td>
</tr>
<tr>
<td>USG ≤ 1.012</td>
<td>92.7 (87.0–96.4)</td>
<td>59.4 (40.6–76.3)</td>
<td>2.28</td>
<td>0.12</td>
<td>90.7</td>
<td>65.5</td>
</tr>
<tr>
<td>USG &gt; 1.012</td>
<td>95.5 (91.4–98.1)</td>
<td>49.4 (41.3–57.4)</td>
<td>1.89</td>
<td>0.09</td>
<td>68.1</td>
<td>90.7</td>
</tr>
</tbody>
</table>

LR− = Negative likelihood ratio. LR+ = Positive likelihood ratio. NPV = Negative predictive value. PPV = Positive predictive value.

See Table 1 for remainder of key.

Table 3—Diagnostic accuracy of the urine dipstick test as evaluated in 507 canine urine samples, with the UPC ratio as the reference criterion for detection of proteinuria and a dipstick-test result considered negative when 0+ or positive when ≥ 2+.

<table>
<thead>
<tr>
<th>Urine sample assessed</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>LR+</th>
<th>LR−</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
<td>70.3 (64.9–75.2)</td>
<td>97.4 (94.0–99.1)</td>
<td>26.8</td>
<td>0.31</td>
<td>97.3</td>
<td>66.4</td>
</tr>
<tr>
<td>USG &lt; 1.000</td>
<td>67.1 (60.9–72.8)</td>
<td>98.3 (94.3–100.0)</td>
<td>63.7</td>
<td>0.33</td>
<td>99.4</td>
<td>53.1</td>
</tr>
<tr>
<td>USG ≥ 1.030</td>
<td>92.8 (71.3–91.1)</td>
<td>95.8 (83.7–98.9)</td>
<td>19.9</td>
<td>0.18</td>
<td>95.9</td>
<td>93.3</td>
</tr>
<tr>
<td>USG ≤ 1.012</td>
<td>56.2 (47.5–64.7)</td>
<td>96.9 (83.8–99.9)</td>
<td>18.0</td>
<td>0.45</td>
<td>98.7</td>
<td>34.1</td>
</tr>
<tr>
<td>USG &gt; 1.012</td>
<td>81.0 (74.5–86.5)</td>
<td>97.5 (93.6–99.3)</td>
<td>32.0</td>
<td>0.19</td>
<td>97.3</td>
<td>81.9</td>
</tr>
</tbody>
</table>

See Tables 1 and 2 for key.

Table 4—Suggested interpretation of urine dipstick-test results to assess proteinuria according to USG in dogs.

<table>
<thead>
<tr>
<th>Dipstick-test result</th>
<th>0+ (0 mg/dL)</th>
<th>1+ (30 mg/dL)</th>
<th>≥ 2+ (≥ 100 mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1.012</td>
<td>NP</td>
<td>UPC ratio (d)</td>
<td>UPC ratio (g)</td>
</tr>
<tr>
<td>&gt; 1.012 and ≤ 1.030</td>
<td>NP</td>
<td>NP</td>
<td>UPC ratio (g)</td>
</tr>
<tr>
<td>≥ 1.030</td>
<td>NP</td>
<td>NP</td>
<td>UPC ratio (g)</td>
</tr>
</tbody>
</table>

(d) = Quantification of urine protein loss with UPC ratio is necessary to diagnose proteinuria. (g) = Quantification of urine protein loss with UPC ratio is necessary to grade proteinuria. NP = Nonproteinuric, quantification of urine protein loss is not required. Only interpretations for urine samples in which the exact amount of proteinuria should be quantified with the UPC ratio are shown.

Bence-Jones proteinuria, particularly multiple myeloma and plasmacytoma (data not shown). It has been suggested that the sensitivity of the urine dipstick test is low in such situations because light chains of immunoglobulins have less free amino groups that can react with the protein test, thereby increasing the yield of false-negative results. However, because plasma cell tumors are rather uncommon in dogs, reliability of results was likely preserved. Furthermore, regarding discordant results between the 2 methods of proteinuria assessment, the possibility of falsely high dipstick-test results must be taken into account. Although urine samples with macroscopic alterations or positive dipstick-test results for substances other than protein had been excluded from the study, one cannot rule out that other interfering substances (eg, drugs or metabolites not measured by the dipstick test) were present and generated false-positive results.

As suggested by the results of the present study, in dogs with a urine dipstick-test result of 30 mg/dL...
(1+), USG ≤ 1.012, and clinical signs consistent with a disease known to cause proteinuria or a known breed predisposition to glomerulopathy, determination of the UPC ratio is important, whereas when clinical signs are not present, the UPC ratio may not need to be assessed. When a urine dipstick test indicates a protein concentration of 30 mg/dL (1+), but the USG is > 1.012, the dog may be considered nonproteinuric and exact quantification of protein loss is not required. Dogs that have urine dipstick-test results that exceed 100 mg/dL (2+) at any urine concentration are likely to be proteinuric, and the loss of protein should be quantified with the UPC ratio. Irrespective of USG, the data reported here indicated a negative urine dipstick-test result (0+) can be used by veterinarians to classify dogs as nonproteinuric, thereby possibly eliminating the need for determination of the UPC ratio.

References