Urine protein-to-creatinine concentration ratio in samples collected by means of cystocentesis versus manual compression in cats

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Objective—To compare urine protein-to-creatinine concentration (UPC) ratios in samples collected by means of cystocentesis versus manual compression in cats.

Design—Evaluation study.

Animals—43 client-owned cats requiring urinalysis.

Procedures—in all cats, 5 mL of urine from the midstream phase of micturition was collected by means of manual compression and, subsequently, an additional 5 mL of urine was obtained by means of ultrasound-guided cystocentesis. A complete urinalysis was performed on all samples, and UPC ratios were determined.

Results—Cats were classified on the basis of the International Renal Interest Society staging system as being free from proteinuria (UPC ratio, <0.2; n = 19) or as having borderline proteinuria (UPC ratio, 0.2 to 0.4; 7) or proteinuria (UPC ratio, >0.4; 17). None of the cats had postrenal proteinuria. A significant linear correlation was identified between UPC ratios in urine samples obtained by means of manual compression and ratios in samples obtained by means of cystocentesis. For all cats, UPC ratios for samples obtained by the 2 collection methods resulted in classification in the same IRIS stage.

Conclusions and Clinical Relevance—Results suggested that collection of a urine sample from the midstream phase of micturition by manual compression would be a reliable alternative to cystocentesis for the determination of UPC ratio in cats, provided that postrenal proteinuria was excluded by means of urine sediment analysis. Once postrenal proteinuria was ruled out, the method used to collect urine samples did not appear to influence the quantification of urine protein concentration. (J Am Vet Med Assoc 2015;246:862–867)

Proteinuria is a general term that describes the presence of any type of protein (albumin, globulins, Bence-Jones proteins, and other proteins) in the urine.1,2 In clinical practice, the term proteinuria generally refers to an abnormal, excessive amount of urinary protein.3,4 Albumin is the principal protein in urine in most healthy and diseased dogs and cats.2,5 Persistent proteinuria with an inactive urine sediment is an important clinicopathologic marker of CKD in dogs and cats.2,6 Previously, proteinuria was primarily considered a marker of glomerular disease and was generally considered to be clinically unimportant unless associated with hypoalbuminemia or a UPC ratio >3.0 in dogs2 or >1.0 in cats.6,10 Presently, persistent renal proteinuria, even at low levels, is known to be a

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risk factors for CKD progression in both species. Sev-
eral investigations have emphasized the importance of renal proteinuria as a diagnostic and prognostic marker and therapeutic target in dogs and cats with CKD and have associated renal proteinuria with increases in morbidity and mortality rates. In addition, the risk of developing adverse outcomes has been found to be related to the magnitude of proteinuria. These findings have led the IRIS to propose that persistent proteinuria is important in staging dogs and cats with CKD. Animals with UPC ratios < 0.2 are classified as not having proteinuria, whereas animals with UPC ratios > 0.4 (cats) or > 0.5 (dogs) are considered to have proteinuria. Animals with UPC ratios between 0.2 and 0.4 (cats) or between 0.2 and 0.5 (dogs) are considered to have borderline proteinuria. Nowadays, if persistent renal proteinuria is suspected or detected, urine protein excretion should be quantified to assess the severity of renal lesions and evaluate the response to treatment or the progression of disease.

Proteinuria may be quantified by determining the total amount of protein excreted in urine over a 24-hour period, calculating the UPC or urine albumin-to-creatinine concentration ratio, or performing a quantitative ELISA to detect microalbuminuria. In human medicine, measurement of 24-hour protein excretion is the gold standard. In veterinary medicine, the most widely used method for quantifying proteinuria is calculation of the UPC ratio. The UPC ratio correlates well with daily protein excretion in clinically normal cats and dogs with experimentally induced CKD, as well as in healthy dogs and dogs with proteinuria. Calculating the UPC ratio is practical because it can be determined on the basis of results for a single urine sample.

The UPC ratio is most frequently determined for urine samples collected by means of cystocentesis and may be contraindicated in some patients, such as those with coagulopathy. In healthy cats and dogs and in cats and dogs with proteinuria, UPC ratios for voided urine samples correlate well with 24-hour protein excretion also determined in voided samples and in healthy cats, UPC ratios for samples collected by means of cystocentesis correlate with daily protein excretion determined in voided urine samples. In dogs, UPC ratios for voided urine samples are similar to ratios for urine samples obtained by means of cystocentesis, thus suggesting that free-catch urine samples can be safely used in clinical practice to quantify severity of proteinuria. Another study involving dogs concluded that urine albumin, N-acetyl-β-D-glucosaminidase, and retinol-binding protein concentrations were similar in voided samples and samples obtained by means of cystocentesis.

To the best of our knowledge, no studies comparing UPC ratios for urine samples collected by means of cystocentesis with ratios for samples obtained by means of manual compression have been performed in cats. The purpose of the study reported here was to determine, in cats, whether UPC ratios for urine samples collected by means of manual compression were similar to ratios for samples collected by means of cystocentesis.

### Materials and Methods

**Animals**—Forty-three client-owned cats requiring urinalysis were included in the study. Cats were either healthy cats undergoing urinalysis during standard preoperative screening or were diseased cats undergoing urinalysis as part of their diagnostic plan. Cats with lower urinary tract or genital diseases, cats with spermaturia, and cats in which manual compression of the urinary bladder was difficult were excluded from the study. Prerenal proteinuria was considered to be unlikely on the basis of serum total protein, albumin, and globulin concentrations and exclusion of hemoly-sis, myoglobinemia, hemoglobinuria, and myoglobin-uria in all cats enrolled in the study. All owners gave informed consent. The study was approved by the scientific council of the Vasco da Gama University School as complying with Portuguese legislation for the protection of animals (law No. 92/1995).

**Urine sample collection and analysis**—In all cats, 5 mL of urine from the midstream phase of micturition was collected by means of manual compression and, ≤ 3 hours later, an additional 5 mL of urine was obtained by means of ultrasound-guided cystocentesis. Immediately after collection, urine samples were centrifuged at 350 × g for 5 minutes, and 4 mL of the supernatant was collected and submitted for determination of the UPC ratio. The remainder of each urine sample was submitted for a complete urinalysis, including measurement of urine specific gravity, evaluation of the sample with a test strip, and sediment analysis. Cats for which results of the sediment analysis were indicative of an active inflammatory process (ie, > 5 RBCs or WBCs/hpf, bacteria, or increased cellularity) or with spermaturia were excluded. Urine protein and creatinine concentrations were determined within 24 hours after sample collection by means of the biuret and Jaffé methods, respectively. Cats were classified on the basis of the IRIS substaging system as being free from proteinuria (UPC ratio, < 0.2) or as having borderline proteinuria (UPC ratio, 0.2 to 0.4) or proteinuria (UPC ratio, > 0.4).

**Statistical analysis**—Whenever available, information on sex, age, breed, living conditions, clinical status, FIV and FeLV infection status, and results of hematologic and serum biochemical analyses and urinalyses were recorded. Continuous data were evaluated by means of the Shapiro-Wilk test to determine whether they were normally distributed. The Spearman rank correlation test (nonnormally distributed data) or Pearson product-moment correlation test (normally distributed data) was then used to determine correlations between UPC ratios obtained in urine samples collected by means of cystocentesis versus manual compression for all cats as a group and for males and females separately. Sensitivity, specificity, true-positive rate, false-positive rate, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were calculated to assess the reliability of the urine dipstick test to detect proteinuria, with UPC ratio considered to be the gold standard test. Statistical analyses were performed with the aid of statistical software. For all analyses, values of $P \leq 0.05$ were considered significant.
Results

The 43 cats included in the study consisted of 34 domestic shorthair cats and 9 Persians. There were 18 males and 25 females. Information on age was available for 40 cats; age of these 40 cats ranged from 6 months to 17 years (mean, 7.26 years; SD, 5.06 years). Nine cats initially enrolled in the study were excluded; 7 were excluded because results of urine sediment analysis were indicative of active inflammation, and 2 were excluded because of spermaturia.

Nineteen cats were classified as being free from proteinuria (UPC ratio, < 0.2), 7 were classified as having borderline proteinuria (UPC ratio, 0.2 to 0.4), and 17 were classified as having proteinuria (UPC ratio, > 0.4). Cats with borderline proteinuria included 5 domestic shorthairs and 2 Persians, of which 3 were male and 4 were female, with ages ranging from 6 months to 11 years (mean, 5.93 years; SD, 3.59 years). Cats with proteinuria included 13 domestic shorthairs and 4 Persians, of which 10 were male and 7 were female, with ages ranging from 1 to 17 years (mean, 6.33 years; SD, 5.49 years). Of the 17 cats with proteinuria, 6 had azotemia (serum creatinine concentration > 1.8 mg/dL), 5 had hypoalbuminemia (serum albumin concentration < 2.3 mg/dL), and 7 had a low (< 1.035) urine specific gravity.

When descriptive statistics were calculated for UPC ratios for all cats as a group and for male and female cats separately, minimum and maximum values, mean values, median values, and interquartile ranges (25th to 75th percentiles) were similar and largely overlapped (Figure 1). For all 43 cats, UPC ratios ranged from 0.02 to 6.99 (mean, 0.82; median, 0.210; interquartile range, 0.100 to 0.740) for samples collected by means of cystocentesis and from 0.03 to 7.03 (mean, 0.86; median, 0.210; interquartile range, 0.110 to 0.770) for samples collected by means of manual compression. For the 25 female cats, UPC ratios ranged from 0.02 to 3.90 (mean, 0.55; median, 0.190; interquartile range, 0.100 to 0.580) for samples collected by means of cystocentesis and from 0.03 to 3.94 (mean, 0.57; median, 0.190; interquartile range, 0.101 to 0.585) for samples collected by means of manual compression. For the 18 male cats, UPC ratios ranged from 0.07 to 6.99 (mean, 1.20; median, 0.495; interquartile range, 0.115 to 1.530) for samples collected by means of cystocentesis and from 0.05 to 7.03 (mean, 1.25; median, 0.525; interquartile range, 0.173 to 1.495) for samples collected by means of manual compression. When UPC ratios for samples obtained by the 2 methods were compared for each cat, this difference was < 0.1 for all but 3 cats, which had differences of 0.13, 0.14, and 1.04.

When values for all 43 cats were considered, there was a significant ($P < 0.001$) linear correlation (Spearman $\rho = 0.987; r^2 = 0.975$) between UPC ratios for urine samples collected by means of manual compression and ratios for samples collected by means of cystocentesis (Figure 2). Similarly, there were significant ($P < 0.001$) linear

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correlations between UPC ratios for samples obtained by means of manual compression versus cystocentesis for both female (Pearson $r^2 = 0.987$; Figure 3) and male (Pearson $r^2 = 0.987$; Figure 4) cats. For all cats, UPC ratios for samples obtained by the 2 collection methods resulted in classification in the same IRIS substage.

Results of the urine dipstick test for proteinuria (with all test results of trace or greater classified as positive) were not significantly correlated with UPC ratio, regardless of whether samples were collected by means of cystocentesis ($P = 0.187$; Spearman $p = 0.216$; $r^2 = 0.046$) or manual compression ($P = 0.223$; Spearman $p = 0.190$; $r^2 = 0.040$). For both urine collection methods, sensitivity of the urine dipstick test (with all test results of trace or greater classified as positive) to detect proteinuria was 81.0% and 85.7% when UPC ratios of $\geq 0.2$ and $> 0.4$, respectively, were used as the cutoff (Table 1). In all instances, specificity was $\leq 28%$.

**Discussion**

Results of the present study suggested that collection of a urine sample from the midstream phase of micturition by manual compression would be a reliable alternative to cystocentesis for the determination of the UPC ratio in cats, provided that postrenal proteinuria was excluded by means of urine sediment analysis. Once postrenal proteinuria was ruled out in the cats in the present study, the method used to collect urine samples did not appear to influence the quantification of urine protein concentration.

In dogs and cats, cystocentesis is, in most situations, the preferred method for collecting urine samples, and the UPC ratio is most frequently determined for urine samples collected by means of cystocentesis. However, cystocentesis has some disadvantages and may be contraindicated in certain patients, such as those with a coagulopathy. Our results indicated that a urine sample obtained by means of manual compression can be used in situations when cystocentesis cannot be performed.

Obtaining a free-catch urine sample from the midstream phase of micturition in cats is difficult, and manual compression of the urinary bladder can be used as an alternative to obtaining a free-catch urine sample. However, this technique must be used carefully because it might be associated with bladder wall trauma or rupture and ureteral reflux, particularly in males. For these reasons, manual compression should only be considered when cystocentesis is not possible to perform or is contraindicated. In the present study, none of the cats developed complications related to manual bladder compression. Nonetheless, some cats initially selected were excluded from the study because manual compression was difficult, precluding collection of urine samples by this method. Most cats excluded for this reason were male.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cystocentesis</th>
<th>Manual compression</th>
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<tbody>
<tr>
<td></td>
<td>UPC ratio $\geq 0.2$</td>
<td>UPC ratio $&gt; 0.4$</td>
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<tr>
<td>Sensitivity (%)</td>
<td>81.0</td>
<td>85.7</td>
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<tr>
<td>Specificity (%)</td>
<td>22.2</td>
<td>24.0</td>
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<tr>
<td>True-positive rate (%)</td>
<td>72.7</td>
<td>72.4</td>
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<tr>
<td>False-positive rate (%)</td>
<td>24.0</td>
<td>22.2</td>
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<tr>
<td>Positive predictive value (%)</td>
<td>54.8</td>
<td>38.7</td>
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<tr>
<td>Negative predictive value (%)</td>
<td>50.0</td>
<td>75.0</td>
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<tr>
<td>Positive likelihood ratio</td>
<td>1.04</td>
<td>1.13</td>
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<td>Negative likelihood ratio</td>
<td>0.86</td>
<td>0.80</td>
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For the urine dipstick test, all results of trace or greater were classified as positive. The UPC ratio was used as the gold standard; 2 cutoffs for a positive result (UPC ratio $\geq 0.2$ and UPC ratio $> 0.4$) were used.
Recently, a study comparing UPC ratios in urine samples collected from dogs by means of cystocentesis versus free catch detected a significant \((P < 0.001)\) linear correlation \((r^2 = 0.90)\) between results obtained with the 2 methods of urine collection. In dogs, collection of urine samples by free catch can be used as a reliable alternative to cystocentesis for determination of the UPC ratios. In the present study, a similar significant linear correlation between UPC ratios in urine samples collected from cats by means of cystocentesis versus manual compression was found. In the previous study, 75 of 81 (92.6%) dogs had UPC ratios for both urine samples that resulted in classification in the same IRIS stage. In the present study, all 43 cats had UPC ratios for both urine samples that resulted in classification in the same IRIS stage. However, in this study, UPC values were calculated to 2 decimal places. If values had been rounded to 1 decimal place, then 2 of the 43 cats would have had UPC ratios for the 2 collection methods that resulted in classification in different IRIS substages. Both of these cats had a UPC ratio for the sample collected by means of cystocentesis of 0.1, indicating a lack of proteinuria, and a ratio of 0.2 (0.15 in one cat and 0.18 in the other) for the sample collected by means of manual compression, indicating borderline proteinuria (false-positive result). As in the study performed in dogs, the major discordance between the 2 methods of collection occurred near the cutoff for classifying animals as having borderline proteinuria, emphasizing the necessity of serial determination of UPC ratios to confirm the persistence of renal proteinuria.

The urine dipstick test in the present study had poor reliability in determining the presence or severity of proteinuria. Results of the urine dipstick test for proteinuria were not significantly correlated with the UPC ratio, and the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio when the dipstick test was used to identify proteinuria were low. These results were consistent with findings of previous studies, indicating that the urine dipstick test has questionable diagnostic value in identifying proteinuria in cats owing to the high rates of false-positive and false-negative results.

In the present study, cats with >5 RBCs or WBCs/μL of hematuria, or increased cellularity in the urinary sediment were considered likely to have an active inflammatory process involving the lower urinary tract and were excluded. In dogs, it has been shown that hematuria only significantly influences results of urine protein quantification when macroscopic hematuria (ie pink or red urine) is present. The same is likely true for cats, and the presence of low-grade hematuria should not be considered a contraindication for calculating the UPC ratio. In the present study, UPC ratios for urine samples obtained by the 2 urine collection methods provided similar clinical information; however, cats with postrenal proteinuria, determined on the basis of urine sediment analysis, were excluded. In fact, postrenal proteinuria is common in cats and dogs, and a complete urinalysis with sediment analysis is needed to rule it out. According to our results, once postrenal proteinuria was excluded, the method used to collect urine samples did not influence the quantification of urine protein concentration. Therefore, we concluded that for cats, collection of urine samples from the midstream phase of micturition by means of manual compression would be a reliable alternative to cystocentesis for the determination of UPC ratios. Nonetheless, it should be emphasized that cystocentesis is the preferred method of urine collection, because contamination from the lower urinary tract is reduced with cystocentesis and proteinuria is associated with fewer risks than manual expression of the bladder, particularly in male cats.

References


From this month’s AJVR

Platelet activation in a population of critically ill dogs as measured with whole blood flow cytometry and thromboelastography

Sean B. Majoy et al

**Objective**—To determine whether critically ill dogs had increased platelet activation and whether the proportion of activated platelets correlated with severity of illness.

**Animals**—92 dogs in the intensive care unit of a veterinary teaching hospital and 24 healthy control dogs.

**Procedures**—Flow cytometry with monoclonal mouse anti-human CD61 and CD62 antibodies in resting and ADP-treated samples and kaolin-activated thromboelastography were used to compare platelet activation in blood samples of critically ill and control dogs. Serum antithrombin, von Willebrand factor, fibrinogen, and activated protein C concentrations; prothrombin time (PT); and activated partial thromboplastin time (aPTT) were measured. Revised survival prediction index, acute patient physiology and laboratory evaluation, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome scores were used to estimate severity of illness. Severity of illness scores and platelet activation measurements were compared with survival time and duration and cost of hospitalization.

**Results**—Critically ill and control dogs had no differences in platelet activation for non–ADP-treated samples measured. Critically ill dogs had significantly increased platelet activation in response to 2, 6, and 10mM ADP. Critically ill dogs had significantly increased maximum amplitude angle, and G (global clot strength) and significantly decreased clot formation time. Critically ill dogs had significantly increased fibrinogen concentration, PT, and aPTT and significantly decreased antithrombin concentration. Survivors and nonsurvivors had similar flow cytometry and thromboelastography values. Three dogs developed macrothrombosis.

**Conclusions and Clinical Relevance**—In this study, critically ill dogs had hyperreactive platelets, which may have contributed to a high incidence of hypercoagulability in this patient population. (Am J Vet Res 2015;76:328–337)