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The effect of inter-laboratory variability on the protein:creatinine (UPC) ratio in canine urine

G. Rossi [a,*], W. Bertazzolo [b], F. Dondi [c], M. Binnella [a], M. Gruarin [c], P. Scarpa [a], S. Paltrinieri [a]

[a] Department of Veterinary Sciences and Public Health, University of Milan, Via Celoria 10, Milano 20133, Italy
[b] Veterinary Animal Hospital Città di Pavia, Pavia, Italy
[c] Department of Veterinary Medical Sciences, Alma Mater Studiorum – University of Bologna, Ozzano dell'Emilia (BO), Italy

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ABSTRACT

Quantification of proteinuria is a fundamental step in staging dogs with chronic kidney disease and in monitoring the course of disease or the efficacy of anti-proteinuric treatments. Analytical precision and accuracy of the proteinuria assessment could be affected by several factors such as biological variability, different operators and quality control materials. The aim of this study was to assess whether inter-laboratory variability could affect the urinary protein to creatinine (UPC) ratio and whether this variability may affect patient classification according to the International Renal Interest Society (IRIS) sub-staging system. The same urine samples were analysed in three different laboratories using different instruments and different reagent brands.

The results of the three laboratories were highly correlated to each other although urinary protein (UP), urinary creatinine (UC) and the UPC ratio of one laboratory were found to be significantly higher than those of the other two. No significant differences between the other two laboratories were recorded. The concordance in classifying dogs according to the IRIS guidelines was good if all three proteinuria categories were analysed separately or if borderline proteinuric (BP) dogs were included in the proteinuric group, and very good if BP dogs were merged into the non-proteinuric group. The inter-laboratory variability in UPC ratio measurement was not so great as to impede the identification of proteinuric dogs, but may influence the estimation of the magnitude of proteinuria.

Introduction

According to the current guidelines (Lees et al., 2005) quantification of proteinuria is a key factor in staging chronic kidney disease (CKD). Proteinuria is an early marker of kidney injury and a risk factor in uraemic crises. The magnitude of proteinuria at first diagnosis correlates with the outcome of the disease and severe proteinuria is associated with faster progression of CKD (Jacob et al., 2005). The calculation of the urinary protein to creatinine (UPC) ratio is used routinely as it is correlated with daily protein excretion and can be assessed in a single randomly collected urine sample (DiBartola et al., 1980; Center et al., 1985; Moore et al., 1991; Lees et al., 2005).

The International Renal Interest Society (IRIS) established a staging system for CKD[1] based on serum creatinine concentration, blood pressure and proteinuria (Elliott and Watson, 2008): the decision limits (cut-off points) for each parameter are species-specific. Nevertheless, pre-analytical, analytical and biological variability could affect the UPC ratio. A day-to-day biological variability has been reported (Nabity et al., 2007) and therefore the demonstration of persistent proteinuria requires at least three different samplings, each separated by a 2–3 week time interval (Lees et al., 2005).

It has been demonstrated that the analytical variability of creatinine measurement using the modified Jaffe method is inversely proportional to the urine specific gravity (USG), and variability in the pyrogallol red method for the measurement of urinary protein concentration (although modest) is inversely proportional to proteinuria (Westgard, 2003; Rossi et al., 2012). Therefore, dogs with borderline proteinuria could be erroneously sub-staged based on the IRIS guidelines (Rossi et al., 2012) and when UPC ratio values are close to the IRIS decision limits, samples should be re-analysed.

Analytical precision and accuracy can be affected by several factors, most of which are evaluated in quality assurance programmes (Gunn-Christie et al., 2012) and routinely estimated using internal and external monitoring and quality controls. A number of variables may affect analytical precision including operator knowledge, quality control (QC) materials and rules, sample handling, instruments and the manufacturer of the reagents, as well as variable sample volumes, automated dilutions, and linearity (European Confederation of Laboratory Medicine, 2000). These variables could affect the precision and accuracy of UPC ratio measurement if runs are performed in different laboratories.

* Corresponding author. Tel.: +39 0250318103.
E-mail address: gabriele.rossi@unimi.it (G. Rossi).

[1] See: http://www.iris-kidney.com (accessed 30 July 2014).

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The aim of this study was to evaluate the canine UPC ratio, to assess if inter-laboratory variability affects the UPC ratio when the same sample is analysed in different laboratories, and to examine whether this variability may in turn affect patient classification according the IRIS sub-staging system.

Materials and methods

Animals and samplings

This prospective study was conducted in three different laboratories (Labs 1, 2 and 3). Samples ($n = 105$) were collected from dogs over a 6 month period (May–November 2013) irrespective of underlying diseases. All canine urine samples enrolled in this study matched the inclusion criteria that the dogs were (1) of any age, breed, gender and size, and (2) were either healthy animals in which urinalysis had been included in the health screening assessment, or dogs with renal, metabolic and/or inflammatory disease(s) that could induce proteinuria.

The presence of active sediment was not an exclusion criterion in this study since our aim was to investigate the analytical variability of UPC measurement, irrespective of the final diagnosis or of the clinical condition.

Analytical methods

Samples were collected by free catch or cystocentesis. Five millilitres of each sample was centrifuged at 500 *g* for 5 min then the supernatant was transferred into three plain tubes (1 mL each) and frozen at –20 °C. The remaining urine was used to resuspend the urine sediment and 50 μL of the resuspended sediment was examined microscopically at 400× magnification to count the mean number of red blood cells (RBCs) and white blood cells (WBCs) per high power field (hpf).

Bacteriuria, spermaturia, lipiduria, and the presence of epithelial cells, casts, and crystals were also evaluated according to a semiquantitative scale (rare, moderate, abundant, or very abundant). Sediment was classified as active on the basis of the presence of one or more of the following findings: bacteriuria, a moderate number of casts, and >5 RBCs, WBCs, or epithelial cells/hpf. Samples with none of these findings were classified as inactive.

Quantification of urinary proteins (UP) and urinary creatinine (UC), and calculations of the UPC ratio were performed in batches, after a maximum storage time of 2 months; it has been shown that during this period of time artefacts that could affect the measurement of proteinuria do not occur (Rossi et al., 2012). Three work sessions were performed to avoid further interference, and analyses were undertaken at the same time in the three different laboratories: aliquots were delivered under controlled temperatures and analysed immediately after arrival.

Instruments, reagents and standard operating procedures (SOP) for each laboratory

In Lab 1 the analyses were performed using an automated spectrophotometer (Cobas Mira, Roche Diagnostics). UPs were measured using pyrogallol red (Total Proteins High Sensitivity, Ben Biochemical Enterprise); this method is linear up to 250 mg/dL, thus in accordance with the SOP of Lab 1, samples with UPs >250 mg/dL were manually diluted 1:5 with distilled water and re-analysed. The UC was measured with a modified Jaffe method (Real Time Diagnostic Systems). Samples were manually diluted 1:20 with distilled water to fit the linearity of the method (Rossi et al., 2012). Occasionally, particularly concentrated urine samples were further diluted to 1:100 to fit the linearity of the method.

Lab 2 analyses were also performed with the Cobas Mira automated spectrophotometer and UPs using pyrogallol red (Real Time Diagnostic Systems). The linearity of this method is up to 150 mg/dL, thus samples with values >150 mg/dL were manually diluted 1:5 with distilled water and re-measured. UC was measured with a modified Jaffe method (Real Time Diagnostic Systems). A manual pre-dilution 1:10 was performed to fit the linearity of the method.

With Lab 3, the analyses were performed using the automated spectrophotometer Olympus AU 400 (Olympus/Beckman Coulter). UPs were measured using pyrogallol red (Urinary/CSF protein OSR6170, Olympus/Beckman Coulter). The linearity of this method is up to 200 mg/dL, so samples were automatically diluted 1:4 and re-analysed if values were higher than this threshold. UC was measured with a modified Jaffe method (Creatinine OSR6178, Olympus/Beckman Coulter); the linearity is up to 400 mg/dL and so samples were automatically diluted 1:10 and re-analysed if values were higher than this threshold.

QC was performed by all three laboratories before any work session. Labs 1 and 2 used two levels (normal and high) of control serums (Lab 1: Normal Control Serum and Pathological Control Serum; Lab 2: Preci Norm H and Preci Path H, Hagen Diagnostica) for creatinine. For UP a specific control with bovine albumin (100 mg/dL) was used; creatinine was also included in a monthly ring test (Monthly Clinical Chemistry Program, Riquas). Lab 3 used specific control material prepared from human urine, with low and high concentrations of creatinine (84 and 154 mg/dL, respectively) and total protein (15.8 and 51.6 mg/dL, respectively) (Quantimetrix-Dropper Urine Chemistry control levels 1 and 2, Sentinel diagnostics).

In each laboratory the UPC ratio was calculated using the formula UPC ratio = [UP]/[UC]. The choice to use metric units instead of SI units throughout the study was driven by the need for a prompt calculation of the UPC ratio; indeed we used the same units (mg/dL) for both UP and UC. Samples were then classified according to IRIS guidelines as non-proteinuric (NP = UPC ratio <0.20), borderline proteinuric (BP = UPC ratio of 0.21–0.50), or proteinuric (P = UPC ratio >0.5).

Statistical analysis

Statistical analysis was performed using the Analyse-it software, version 2.21. A P value < 0.05 was considered statistically significant. Normality was tested with the Kolmogorov–Smirnov test. UP, UC and UPC values obtained in the three laboratories were compared to each other with the Friedman non parametric ANOVA paired test followed by a Wilcoxon signed rank test (only performed if the Friedman tests delivered a significant result) to compare the results between individual laboratories. The correlation between individual laboratories was compared to paired sets of data using the Spearman correlation test and the agreement between pairs of laboratories was assessed by Passing Bablok and Bland–Altman tests.

The concordance in classifying samples in the three classes (NP, BP and P) defined by the IRIS guidelines was evaluated using the Cohen's kappa (κ) concordance test. Concordance analysis was then repeated, firstly including the BP dogs in the NP group to assess the ability of each method to identify the true proteinuric dogs, and secondly including BP dogs in the P group in order to assess the ability of each method to identify the non proteinuric dogs. Results of Cohen's κ concordance tests were classified as very good (κ = 0.8–1), good (κ = 0.6–0.8), moderate (κ = 0.4–0.6), fair (κ = 0.2–0.4), or poor (κ values < 0.2) (Landis and Koch, 1977).

Results

One hundred and five urine samples were collected (30 from Lab 1, 22 from Lab 2, 53 from Lab 3), from 105 dogs of different ages and breeds including 55 samples collected by free-catch and 50 by cystocentesis. Twenty-six samples had active sediment (12 free catch, 14 cystocentesis), while in 79 cases (43 free catch, 36 cystocentesis) the sediment was inactive.

Samples were classified by Lab 1 (randomly selected to estimate the proportion of samples belonging to the three IRIS substages) as NP ($n = 52$), BP ($n = 17$), and P ($n = 36$). Median UP concentration was 17.6 mg/dL (min–max range: 0.0–676.0 mg/dL); median UC concentration was 82.5 mg/dL (min–max range: 7.4–322.0 mg/dL), and median UPC ratio was 0.2 (min–max range: 0.0–12.3). According to lab 1, the UP recorded in samples collected by free catch (median: 24.2, min–max range: 0.0–386.7) was not significantly higher ($P = 0.0709$) than the UP recorded in samples collected by cystocentesis (median: 12.7; min–max range: 0.0–676.0); conversely the UPC ratio was significantly higher ($P = 0.015$) in samples collected by free catch (median: 0.37; min–max range: 0.0–8.6) compared with samples collected by cystocentesis (median: 0.11; min–max range: 0.0–12.3). Both the UP (median: 68.4; min–max range: 0.0–676.0) and the UPC ratio (median: 0.86; min–max range: 0.0–12.3) recorded in samples with active sediment were significantly higher ($P = 0.006$ and $P = 0.009$, respectively) than the UP (median: 15.5; min–max range: 0.0–244.2) and the UPC ratio (median: 0.1; min–max range: 0.0–8.6) recorded in samples with inactive sediment.

Comparison of results recorded in the three laboratories

The UP and the UC values assessed by Lab 3 were significantly higher than those measured by the other two laboratories ($P < 0.001$ for both the analytes and both laboratories) (Table 1). No differences in the measurements of Lab 1 and Lab 2 were recorded. The UPC ratio reported by Lab 3 was significantly higher than that reported by Lab 1 ($P < 0.001$) and Lab 2 ($P < 0.05$).

The results of the three laboratories were highly correlated to each other ($P < 0.001$ for all of the correlation tests). Correlation coefficients for UPs were 0.93 between Labs 1 and 2, 0.94 between Labs 1 and 3, and 0.93 between Labs 2 and 3; correlation coefficients for UC were 0.97, 0.98, and 0.98, respectively; correlation coefficients for the UPC ratio were 0.86, 0.88, and 0.90.