

Are urea and creatinine values reliable indicators of azotaemia in canine babesiosis?

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ABSTRACT

Serum urea and creatinine are extensively used as parameters to screen for azotaemia. Their reciprocal plots roughly correlate with glomerular filtration rate (GFR). They are, however, subject to influence by non-renal factors and to increase their specificity they are often tested concurrently. In renal disease they are expected to behave similarly, with both parameters increasing as GFR decreases. Haemolysis, as it occurs in canine babesiosis, may cause non-renal elevations in serum urea, possibly due to ammonia loading. Furthermore, haemolysis with its related elevations in serum bilirubin and serum haemoglobin, may negatively bias the measurement of serum creatinine due to interference of these substances with the chemical analysis of serum creatinine. This negative bias occurs when the alkaline picrate method, or when direct enzymatic methods based on the measurement of hydrogen peroxide, are used. In order to investigate the significance of these perturbations in canine babesiosis, paired values of serum urea and serum creatinine from *Babesia canis*-negative, non-haemolysis dogs (Group 1), were used to establish a relationship between urea and creatinine over a range of azotaemia by linear regression analysis. This relationship was then used to predict serum creatinine values from actual serum urea values in *B. canis*-positive dogs (Group 2). The mean of the predicted serum creatinine values for Group 2 (237.03 $\mu\text{mol/l}$) was then compared with the mean of the actual serum creatinine values for Group 2 (131.31 $\mu\text{mol/l}$). For Group 2, the mean actual serum creatinine demonstrated a significant negative bias relative to the mean predicted creatinine value. There was also a higher correlation between serum urea and serum creatinine in Group 1 than in Group 2. These findings may have been caused by either nonrenal elevations of serum urea values or by interference with the measurement of serum creatinine. Therefore, although it is possible that some Group 2 dogs with *B. canis* with high serum urea and normal, low, or zero values for serum creatinine were not azotaemic, it is also possible that other Group 2 dogs with these biochemical findings did in fact have azotaemia. This study concluded that urea and creatinine do not behave in a similar and predictable manner over a range of azotaemia in canine babesiosis and are therefore not ideally suited for the detection of renal disease in this clinical setting.

Key words: ammonia, dog, haemoglobin, interference, non-renal elevations.

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INTRODUCTION

Canine babesiosis in South Africa is caused by the protozoan parasite *Babesia canis rossi* and is transmitted by the tick *Haemaphysalis leachi*^{9,11,21}. The main pathogenesis of uncomplicated canine babesiosis involves a haemolytic anaemia. However, when the pathological changes cannot be explained directly by haemolytic anaemia alone, the dog is considered to

have complicated babesiosis. An example of this would be renal failure. Although renal failure is not a common complication, when it occurs it is often fatal^{12,14}.

Some evidence of renal involvement in canine babesiosis can be obtained from urine analysis, including renal tubular epithelial cells (RTE celluria), tubular casts and inappropriate urine specific gravity^{12–14}. Further evidence of renal disease includes azotaemia in the presence of inadequately concentrated urine or *post mortem* findings of acute renal lesions^{3,7,8,15,17}. To document renal failure in babesiosis requires serial evaluation of urine volume, urine analysis and the degree of azotaemia^{12,14}. Dogs with acute renal failure secondary to babesiosis

usually have anuria or oliguria, which fails to respond to adequate rehydration therapy^{12,14}.

Traditionally, biochemical evidence of renal insufficiency is sought from elevations in serum urea and creatinine^{3,4,7,17}. Their reciprocal plots roughly correlate with glomerular filtration rate (GFR)⁷. As the kidney fails, the glomerular filtration rate decreases. A physiological measurement of GFR is in fact considered the most sensitive and specific global marker for altered renal function^{3,4,7}. In the absence of pre- and post-renal causes and provided the biochemical analysis of these analytes is accurate, these are simple and valuable analytes to measure in order to detect renal disease in the form of reduced GFR⁷. Both urea and creatinine require about 60–75 % loss of nephron function before they become elevated^{3,4,7,23}. To increase their specificity, serum urea and creatinine are often tested concurrently. In renal disease they are expected to behave similarly with both parameters increasing as GFR decreases. Serum urea and creatinine are, however, sensitive to significant influences by parameters unrelated to GFR, many of which are encountered in the clinical setting of canine babesiosis; these include ammonia loading with respect to urea, and serum bilirubin and serum haemoglobin interference with respect to creatinine^{12,13,17}.

It has been hypothesised that ammonia loading occurs in canine babesiosis because of haemolysis, blood transfusions and/or gastrointestinal haemorrhage¹⁷. This could lead to a non-renal-related elevation in urea values. Urea is also subject to other non-renal influences. Elevations in serum urea may be caused by a recent protein meal or increased tubular reabsorption during low tubular urine flow rates⁷. A recent protein meal, however, is an unlikely event in canine babesiosis patients because of appetite depression. Decreases in serum urea may be seen due to decreased production in chronic liver disease, decreased protein intake, non-renal excretion in vomitus, and losses in faeces during diarrhoea⁷, all of which may be present in dogs with *B. canis* infection¹⁴.

The creatinine load on the kidney is

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influenced by muscle mass. Creatinine undergoes weak tubular excretion in the male dog and a portion of it is excreted into the intestinal lumen and degraded by intestinal bacteria, providing a non-renal exit from the body⁷. Creatinine measurements are subject to interference by lipaemia, non-creatinine chromagens, haemoglobin and haemoglobin breakdown products^{1,2,5,6,10,18,19,22,23}. In a study testing the influence of bilirubin on the measurement of creatinine, no significant interference was noted in methods that included removal of proteins or in an enzymatic method involving NADH oxidation. However, a heavy negative interference was observed in an alkaline picrate method, and in direct enzymatic methods based on hydrogen peroxide measurement^{6,22}. In another study comparing the effects of interference on the kinetic Jaffé reaction and an enzymatic colorimetric test for creatinine concentration in dogs, serum bilirubin caused a negative bias in both tests while serum haemoglobin had little or no influence on the tests¹⁰.

The current method of creatinine determination at the Section of Clinical Pathology, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria is based on the kinetic modification of the Jaffé alkaline picrate reaction. This method, although insensitive to the influences of non-creatinine chromagens, is sensitive to the effects of haemolysis, bilirubin and lipaemia^{10,22}.

The current method of creatinine determination at a leading private veterinary laboratory is the direct enzymatic method based on the measurement of hydrogen peroxide. There are, however, pigment binders included in this method. This study did not include samples tested by this method.

As mentioned above, haemoglobinaemia and bilirubinaemia are associated with *B. canis* infection. Therefore, the interference that they are reported to cause with the measurement of creatinine^{1,2,5,6,10,17,18,19,22}, may be significant in canine babesiosis. The serum bilirubin and serum haemoglobin values tested in one study ranged from 0–1000 µmol/l and 0–4 g/l, respectively¹⁰. Bilirubin and free haemoglobin levels in dogs with babesiosis can range from 0 to 707 µmol/l and 0 to 5.5 g/l, respectively (Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, unpubl data). In a study where haemoglobin was infused into experimental dogs, a decline in creatinine levels with increasing haemoglobin levels was observed¹³.

The purpose of this study was to evaluate serum urea and creatinine levels in dogs with naturally occurring babesiosis and to ascertain whether the serum urea and creatinine would behave in a similar and predictable manner over a wide range of azotaemia.

MATERIALS AND METHODS

Model

Electronically stored patient data at the Section of Clinical Pathology, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria reflecting paired results of both serum urea and serum creatinine, were used in this retrospective study. Approximately 7000 recorded cases were retrieved. Inclusion and exclusion criteria were applied to the data in order to divide them into the following two groups:

Group 1: dogs were excluded if they were *B. canis*-positive, positive for any other blood parasite, if they had a packed cell volume <35 %, a reticulocyte count >2 %, icterus and/or known hyperbilirubinaemia, known haemoglobinuria and/or haemoglobinaemia, a positive in-saline agglutination test or lipaemia. This group was considered free of haemolytic diseases.

Group 2: dogs were included if they were positive for *B. canis* on blood smear and had paired results for serum urea and creatinine. Dogs were excluded if they were positive for any other blood parasite, or had lipaemia. This group formed the *B. canis*-positive group.

Biochemical tests

Urea and creatinine were determined on a Technicon RA 1000 system (Technicon Instruments Corporation, Tarrytown, USA). Serum urea determination was based on the enzymatic method of Talke and Schubert, using the Technicon method for the RA-1000 analyser²⁰. Serum creatinine determination was based on the kinetic modification of the Jaffé alkaline picrate reaction using the Technicon

method for the RA-1000 analyser¹⁸.

Data analysis

The data were tabulated in a spreadsheet (Excel, Microsoft Corporation, Redmond, WA, USA).

Statistical analysis was performed with Sigma Stat (Jandel Corporation, San Rafael, CA, USA). Descriptive statistics were used to describe the data. A Kruskal-Wallis one-way analysis of variance on ranks was used to test for statistical differences between groups. Pearson's product-moment correlation was used to determine correlation between variables. The level of significance was set at $P < 0.05$.

A regression analysis of the serum urea on serum creatinine values was applied to Group 1 to establish a linear relationship, $y = a + bx$, for these paired results, where y = creatinine, x = urea, and a and b are constants for the specific data set. This equation could be applied to predict serum creatinine values from serum urea values, 'urea-predicted creatinine values' in patients with varying degrees of azotaemia in the absence of serum substances known to interfere with creatinine analysis¹⁶.

The equation $y = a + bx$ for Group 1 was used to predict the mean creatinine value from the actual urea value in Group 2. Scatter plots for Groups 1 and 2 were generated. The mean 'urea predicted serum creatinine' was compared to the mean actual creatinine in Group 2.

RESULTS

Group 1 comprised 3691 samples. The descriptive statistics for the serum urea and creatinine values in Group 1 are summarised in Table 1. The scatter plot for serum urea on serum creatinine in Group 1 and the regression line, represented by the equation $y = a + bx$, are represented in Fig. 1. For Group 1, $a = 45.39419$, $b = 10.01224$, y = urea-predicted creatinine value, x = the actual urea value, and $P = 0.006$. The regression line represents a 'best fit', with data points equally divided above and below the line.

Table 1: Descriptive statistics for the serum urea and creatinine values in Groups 1 and 2.

	Group 1		Group 2	
	Urea (mmol/l)	Creatinine (µmol/l)	Urea (mmol/l)	Creatinine (µmol/l)
No. of samples	3691	3691	397	397
Mean	8.52	130.74	19.14	131.31
Median	5.6	106.00	14.1	128.55
Standard error	0.17	2.09	0.82	6.47
Minimum	0.90	15	1.4	0
Maximum	127.50	1947	117.5	782
10th percentile	3.1	75	4.82	36
90th percentile	14.5	168	40.9	275

Group 2 comprised 397 samples. The descriptive statistics for the serum urea and creatinine values in Group 2 are summarised in Table 1. The scatter plot for serum urea on serum creatinine in Group 2 and the regression line derived from Group 1, are given in Fig. 2. Most of the data points in Fig. 2 fall below the regression line of Group 1. The mean actual serum creatinine for Group 2 was $131.31 \mu\text{mol/l}$. The mean predicted serum creatinine for Group 2, as determined from the mean serum urea from Group 2, was $237.03 \mu\text{mol/l}$.

There was a statistically significant difference between the mean actual serum creatinine value and the mean urea predicted serum creatinine value for Group 2 ($P < 0.001$) (Fig. 3). There was a good correlation between the individual values for actual creatinine and the urea predicted creatinine for Group 1 ($r = 0.89$). There was poor correlation between the individual values for actual creatinine and the urea predicted creatinine for Group 2 ($r = -2.63$).

DISCUSSION

The negative bias displayed in Group 2 for the actual serum creatinine values relative to the urea predicted values was not unexpected given the reported interference by haemoglobin and bilirubin on the measurement of serum creatinine by the picrate method^{1,2,5,6,10,17,18,19,22}. This negative bias may also be caused by nonrenal elevations of the serum urea¹⁷. Thus although the question of which perturbation is most important in this clinical setting cannot be answered by this retrospective study, the results do indicate that urea and creatinine do not behave in a similar and predictable manner over a range of azotaemia in canine babesiosis and are therefore not ideally suited for the detection of renal disease in this clinical setting as they are in non-babesia patients. At this time only a few recommendations can be made. First, one can question the laboratory on the method used to analyse serum creatinine. As mentioned above, methods requiring the removal of protein and the NADH oxidation method are free of interference with regard to the measurement of serum creatinine. Second, interpret discrepancies between urea and creatinine carefully, taking serum pigmentation, serum bilirubin and haemoglobin levels, urine analysis findings, urine production, clinical findings and serial evaluations of renal parameters into account.

CONCLUSION

In conclusion serum urea and serum creatinine do not behave in a similar and

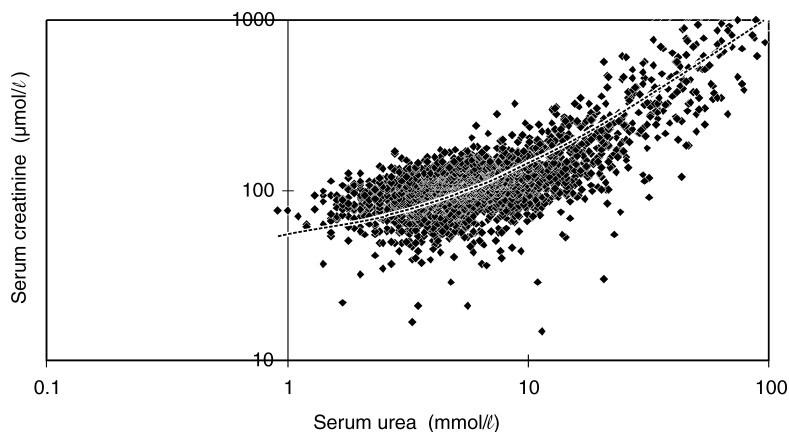


Fig. 1: Scatter plot for Group 1 (*Babesia canis*-negative patients) of serum creatinine on serum urea. The dashed line represents the relationship established for Group 1, serum urea and serum creatinine. A logarithmic scale is used.

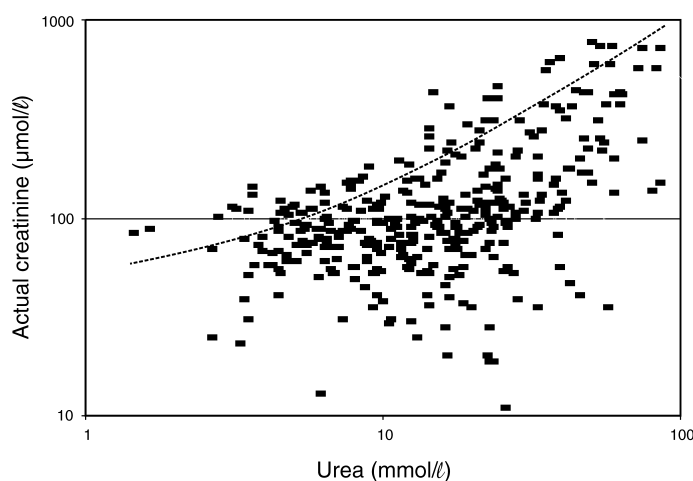


Fig. 2: Scatter plot for Group 2 (*Babesia canis*-positive patients), serum urea on serum creatinine (black squares). The dashed line represents the relationship established for urea and creatinine in Group 1. A logarithmic scale is used.

predictable manner over a range of azotaemia in canine babesiosis, as they do in non-babesiosis patients. They therefore may not reflect the presence of azotaemia and possibly renal disease accurately in

some babesiosis patients. Until alternatives are available they should be interpreted with caution and backed up by ancillary tests and/or serial evaluations of renal parameters in this clinical setting.

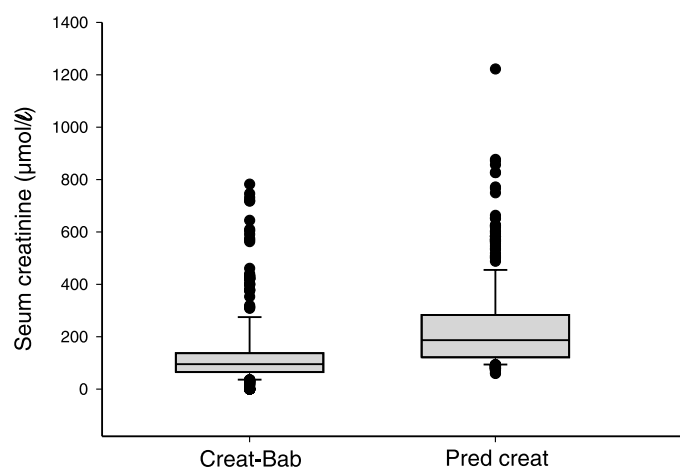


Fig. 3: Serum creatinine concentrations in plasma of 397 dogs with babesiosis (Creat-Bab), and the predicted creatinine (Pred creat). Data are shown as median (horizontal line within box), 25th and 75th percentiles (horizontal ends of boxes), and 10th and 90th percentiles (T-bars). Black dots represent outliers. There was a statistically significant difference between the two groups.

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