Comparison of total protein measurement by biuret method and refractometry in canine and feline plasma

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SUMMARY

The measurement of plasma protein concentration is one of the most frequent laboratory analyses in veterinary medicine. It is usually performed by the biuret reaction in laboratories and bench-top veterinary analyzers. Refractometry is faster, less expensive and suitable for emergency use, but its accuracy is controversial. The aim of this study was thus to compare the results of plasma protein determinations by biuret reaction and refractometer in canine and feline plasma.

The total plasma protein concentration in the plasmas of 156 dogs and 84 cats was determined with a Konelab analyzer and a clinical refractometer. The presence of haemolysis, lipaemia and icterus was recorded. Results were compared by Passing-Bablock agreement, difference plots and by the clinical outcome, i.e. classification of the results as normal or abnormal based on the limits of the reference interval.

The correlation between biuret and refractometry results in plasmas with no visible abnormality was high and the mean differences between the two methods were 6 g/L and 2 g/L in dog and cat plasma respectively. The bias was higher in haemolysed and lipaemic plasmas. The classification of clear plasmas as normal or abnormal was identical, but the ratio of misclassification was higher in haemolysed and lipaemic plasmas.

Refractometry can be a good substitute method for measuring plasma protein concentration in dogs and cats when the plasmas show no visible abnormality but different readings can be observed in haemolysed and lipaemic plasmas.

Keywords : dog - cat - proteins - plasma - biuret - refractometer.

RÉSUMÉ

Comparaison de la mesure des protéines plasmatiques du chien et du chat par la technique du biuret et par réfractométrie. Par A. BRIEND-MARCHAL, C. MÉDAILLE et J.P. BRAUN.

La mesure de la concentration des protéines plasmatiques est l’une des analyses les plus fréquentes en médecine vétérinaire. Elle est principalement effectuée par la réaction du biuret dans les laboratoires d’analyse et les analyseurs des cliniques vétérinaires. La réfractométrie est plus rapide, moins coûteuse et bien adaptée à l’urgence mais son exactitude est controversée. Le but de cette étude était donc de comparer les résultats de dosages des protéines plasmatiques du chien et du chat par la réaction du biuret et par réfractométrie.

La concentration en protéines totales a été déterminée dans le plasma de 156 chiens et 84 chats par un analyseur Konelab et un réfractomètre clinique. La présence d’hémolyse, d’ictère ou de lipémie a été notée. Les résultats ont été comparés par régression de Passing-Bablock, des diagrammes de différence et l’interprétation clinique, c’est-à-dire la classification des résultats en normaux ou anormaux en se fondant sur les limites de l’intervalle de référence.

Dans les plasmas sans anomalie visible, la corrélation entre le biuret et la réfractométrie était élevée, cette dernière donnant des résultats plus élevés en moyenne de 6 g/L et 2 g/L respectivement chez le chien et le chat. La biais était plus important dans les plasmas hémolysées et lipémiques.

La réfractométrie peut être une bonne alternative pour le dosage des protéines totales plasmatiques du chien et du chat dans les spécimens sans anomalie visible ; des résultats différents peuvent être observés dans les plasmas lipémiques ou hémolysées.


Introduction

The measurement of serum/plasma protein concentration is one of the most frequent routine analyses performed to investigate hydroelectrolytic disorders, inflammatory or infectious diseases, colostrum intake, tumours, etc. Its determination is also a prerequisite of protein electrophoresis (for a review, see KANEKO [9]).

The routine measurement of plasma total protein concentration (P-Protein) is commonly performed by biuret method based on the formation of copper chelates by the elozined peptide bonds of proteins at alkaline pH (reviewed in [7, 8, 12]). Although the biuret reaction has been a candidate reference method since the early 1980s [3, 4] it has not been selected as such to our knowledge. The biuret reaction can be performed in liquid reagents or with “dry chemistry” reagents. The latter are the most frequently used in the analyzers of veterinary clinics. Another P-Protein measurement often used in veterinary clinical pathology is based on refractometry. It is often performed in clinical settings lacking advanced equipment for a first evaluation of dehydrated patients : the supernatant of a microhaematocrit tube is deposited on the refractometer for rapid protein measurement, thus allowing rapid comparison of haematocrit and P-Proteins. Refractometry measures the refractive index of a solution, which is mostly dependent on the total protein concentration for plasma [15]. It is rapid and low cost. However, interferences by non protein components such as glucose, cholesterol, etc. have been reported (reviewed in [6]).
In addition, the results of the biuret and refractometry techniques have been found to differ in dogs and other species [6, 13], making the transferability of results difficult and leading to duplicate measurements when the animals have been referred from another clinic or to confusing interpretations when the first result obtained from a haematocrit tube does not agree with a second analysis performed with a conventional analyzer. In a study, the biuret measurements in dog and cat plasmas, were about 5 and 6 % higher than with refractometry but the correlation between the two measurements was high, r = 0.85 and 0.87 in the dog and cat respectively. However, the protein concentrations were "normal", mostly between 50 and 80 g/L, and possible interferences were not evaluated [10].

The aim of this study was thus to compare the P-Protein results obtained in heparin plasmas of dogs and cats by a biuret technique measured with an automated chemistry analyzer in quality controlled conditions and the results obtained on the same day in the same samples by refractometry, and to study possible effects of haemolysis, lipaemia and icterus.

Material and methods

The comparison was carried out using heparin blood samples sent to the laboratory (Vébiotel, Arcueil, France) from mid-October to mid-December 2004. These were centrifuged for 5 minutes at 3500 g (Jouan B4i, Cergy, France) within 2 hours of reception at the laboratory but the delay after sampling was unknown. The plasmas were separated and stored at 4°C until analysis within 2 hours.

Only 63 % and 55 % of the 156 dog and 84 cat plasmas respectively showed no visible signs of haemolysis, icterus or turbidity when observed by the same person (ABM) (Table I). Proteins were analyzed by a biuret technique on a Konelab analyser (Konelab 20i, Thermo, Cergy, France) according to the Société Française de Biologie Clinique method at 37°C, with commercially available reagents (Total Protein, Thermo, Cergy, France). Calibration was performed with two calibrators (Thermo, Cergy, France; Calibrators 1, & 2). Quality control was performed at 2 different concentrations (Abtrol & Nortrol, Thermo, Cergy, France; 51 and 64 g/L respectively). Coefficient of variation (CV) of between-series imprecision were 2.9% and 2.4% (n = 9) at target concentrations of 51 and 64 g/L respectively and the means were 50.3 and 64.3 g/L. A 1/10 pre-dilution of samples was automatically performed when haemolysis or turbidity was detected.

The refractometry measurements were obtained with an Atago (SPR-T2 Model, Fischer Bioblock, France) non temperature-compensated refractometer. The instrument was blanked with distilled water before each series of measurement. All readings were made at room temperature (approximately 20°C) by the same person (ABM). Maximum imprecision was one interval of the scale, i.e. 2 g/L or about 3 % for a mean P-Proteins value of 60 g/L.

The results were compared by Passing-Bablock’s regression analysis, difference plots, ANOVA, Student’s t-test or Mann Whitney’s test according to homogeneity of variances. Calculations were done with an Excel spreadsheet and the Analyze-it set of macroinstructions (Analyze-It Software, Leeds, UK).

Results

OVERALL COMPARISON

P-Proteins concentrations of canine and feline plasmas measured by the biuret technique ranged from 33 to 88 g/L and from 43 to 117 g/L respectively. The overall comparison of results (figure 1) shows that the correlation between the 2 methods was moderate, 0.632 and 0.826 in dog and cat plasma respectively and that the results obtained by refractometry were significantly higher in both species (paired Student’s t-test ; P < 0.001). A higher correlation between the results (r = 0.841 and 0.847) was only obtained for plasmas with no macroscopic abnormalities.

COMPARISON IN PLASMAS WITH NO VISIBLE ABNORMALITY

The results obtained by refractometry in the plasmas with no visible abnormality were higher than those obtained by biuret technique, as shown by the following Passing-Bablock’s equations (95% confidence interval between brackets) (figure 1):

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Haemolysis</th>
<th>Lipaemia</th>
<th>Icterus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>22</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Dog</td>
<td>37</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

Table I. — Number and visual aspects of canine and feline plasmas used in the comparison of P-Protein measurement by biuret method and clinical refractometer (+++++: semi-quantitative evaluation of abnormality).
COMPARISON OF TOTAL PROTEIN MEASUREMENT IN CANINE AND FELINE PLASMA

Dog:
P-Proteins\textsubscript{Refrac} = 1.30 (1.17/-1.44) \cdot P-Proteins\textsubscript{Biuret} - 13.9 (-22.8/-5.5)

Cat:
P-Proteins\textsubscript{Refrac} = 1.22 (1.03/-1.50) \cdot P-Proteins\textsubscript{Biuret} - 13.9 (-34.0/-0.6)

The mean differences between the two techniques were 5.8 and 2.2 g/L in dogs and cats respectively. The differences between the two series of measurements showed both a constant and a proportional bias but the differences of biases between the first and last quartiles were not statistically significant (Mann-Whitney test, P> 0.05). Seventy-one per cent of the results obtained with the 2 techniques in canine plasmas and 100 % of those for feline plasmas were within analytical variability (± 1.96 \cdot (s^2\textsubscript{biuret} + s^2\textsubscript{refrac})^{0.5}).

EFFECTS OF HAEMOLYSIS, ICTERUS, AND LIPAEMIA

Icterus had little effect on the differences observed. Lipaemia and haemolysis increased the differences and the range of results (Table II). Only 44 and 58 % of the results obtained with the 2 techniques in dogs and cats respectively were within 2 CVs. The intensity of lipaemia and haemolysis had no significant effect on the differences between biuret and refractometer measurements.

EFFECTS OF THE (BIURET - REFRACTOMETER) DIFFERENCE ON THE MEDICAL CLASSIFICATION OF DATA

The results were classified as “normal”, “low” and “high” based on the reference interval of the laboratory, i.e. 60-80 g/L in both species (Table III). The percentages of plasmas with no visible abnormality that were classified identically were very similar, whereas the percentage that was classified differently was higher in samples with icterus, haemolysis or lipaemia, especially in dogs.

Discussion

To our knowledge, few studies have been carried out to investigate the relationship between biuret and refractometer
TABLE III. — Effects of the method of protein measurement on the clinical classification of results according to the reference limits (60-80 g/L) and the quality of plasma specimens. (Results as percent of specimens in each class ; < below the lower reference limit ; [ ] within the reference limits ; > higher than the upper reference limit).

<table>
<thead>
<tr>
<th></th>
<th>All plasmas</th>
<th></th>
<th>No visible abnormality</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;</td>
<td>[ ]</td>
<td>&gt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>Dog</td>
<td>Biuret</td>
<td>38.8</td>
<td>62.5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Refractometer</td>
<td>19.7</td>
<td>71.7</td>
<td>11.2</td>
</tr>
<tr>
<td>Cat</td>
<td>Biuret</td>
<td>11.9</td>
<td>67.9</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>Refractometer</td>
<td>11.9</td>
<td>59.5</td>
<td>28.6</td>
</tr>
</tbody>
</table>

FIGURE 2. — Difference diagrams of P-Protein measurement by biuret technique and clinical refractometer in 156 dog (a) and 84 cat (b) plasmas according to the biuret results ( ● = no visible abnormality ; ○ = haemolysis ; □ = lipaemia ; ◊ = icterus).

FIGURE 3. — Effects of icterus, lipaemia and haemolysis on the difference of P-Protein measurements obtained by biuret technique and clinical refractometer in dog (a) and cat (b) plasmas (* : P < 0.05; horizontal bar is the median).
measurements of P-Proteins (for a review in animals, see [5, 6]). A high correlation ($r = 0.97$) was reported in human spec-
cimens, and the interfering effects of cholesterol and urea were
significant when higher than 7.7 and 8.2 mmol/L respectively [11].
The correlation was also high ($r = 0.955$) in horse serum, but lower values were obtained by refractome-
ter than by biuret with a mean difference of 7 g/L [1]. Except
for the study reported in the introduction, comparisons of
biuret and refractometry measurements in dogs and cats have
involved the pleural and peritoneal fluids, in which the cor-
relation between the two techniques is high [2,5,14].

The specimens in this study were selected solely on the
basis of their availability. Breed, age, gender, and disease
status were not taken into account, in order to reproduce the
conditions of routine veterinary practice as accurately as
possible. The only pre-analytical variable taken into account
was the color of the sample due to the frequency of haemo-
lysis, especially in canine plasmas.

The results obtained by refractometry in this study were
higher than those obtained by biuret. The contrary has been
reported in dogs and cats [10] and horse [1] although results
exceeding those obtained by biuret by almost 2 g/L have also
been obtained by refractometry in dogs [17].

The correlation between biuret and refractometer measure-
ments was good in specimens with no visible abnormality.
However, it was not as good as would be expected from 2
techniques for measuring the same analyte. About 30 % of
the variations in the results obtained by refractometry could
not be accounted for by corresponding changes in the biuret
results.

More differences were observed when haemolysis or
lipaemia were present, whereas differences due to icterus
were less intense. In cases of intense lipaemia or haemolysis,
the refractometer reading was less accurate as the separating
line was somewhat blurred. Incorrect visualisation of the
demarcation line was reported with haemolysis before any
factitious increases in plasma protein readings [16].
However, as no satisfying specimen blanking can be done on
icteric, lipaemic or haemolysed plasmas [7], it is not possible
to know which of the two techniques is more accurate.
The manufacturer of the Konelab analyzer indicates that inter-
ferences cannot be detected when the concentrations are lower
than 580 mg/L for bilirubin, 3 g/L for hemoglobin and 1 g/L
for lipids.

The differences between the biuret and refractometer mea-
surements were within the analytical variability in about 3/4
of canine samples and in all feline samples without any
visible abnormality. However, large differences could be
observed without obvious analytical explanation even in
good quality specimens. This was unlikely to be due to the
relative proportions of albumin and globulin but the possible
effects of other plasma analytes such as urea, glucose, cho-
esterol, which have been reported to produce falsely eleva-
ted results by refractometry, could not be assessed.

From a clinical point of view, the main result of this study
was that only a few cases of canine and feline plasmas with
no visible abnormality were not classified identically by
refractometry and biuret. In contrast, the proportion of fal-
sely high results by refractometry or falsely low results by
biuret was high in canine plasma with haemolysis and lipae-
mia, but limited in feline specimens.

It can be concluded from this study that refractometric
measurement of P-Proteins provides a rapid, inexpensive
alternative to biuret determination and can be used with
confidence for canine or feline plasmas without any visible
abnormality. It should however be used with caution in hae-
molized, lipaemic and icteric specimens, especially in dogs,
because of the high frequency of falsely increased results.

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