Background and significance

Hypoproteinemia frequently occurs in critically ill patients and can cause complications related to a decrease in colloid osmotic pressure (COP). Hypoproteinemia may be due to gastrointestinal losses, urine losses, effusions, decreased liver production or increased catabolism. Colloid osmotic pressure is created by large molecules, mostly plasma proteins, within the vascular space that draw fluid towards them maintaining blood volume. If intravascular COP acutely decreases, fluid moves out of the vascular space, therefore reducing circulating blood volume and promoting interstitial oedema. Low COP has been associated with increased development of pulmonary and peripheral oedema and increased mortality in critically ill humans. TPP is monitored closely in critically ill patients as an estimation of intravascular COP and to monitor fluid balance and protein losses.

Artificial colloid fluids are often used to maintain circulating blood volume in dogs with hypoproteinemia. These fluids contain large starch or gelatine molecules that stay within the intravascular space, thus increasing COP and maintaining intravascular blood volume for a longer duration compared to crystalloid fluids. Compared to natural colloids, such as albumin solution and fresh frozen plasma, artificial colloid fluids are less expensive, more readily available, less likely to cause immune-mediated reactions and tend to have a higher COP.

Methods of protein measurement

In veterinary practice, TPP can be measured by two different methods; hand-held refractometry (TPPref) and the biuret technique (TPPbiuret). Refractometers are inexpensive, easy to use and are a point-of-care test commonly used in all types of veterinary practice. The measurement of TPP by the refractometer is based on refraction of light by large molecules and relies on the premise that the majority of large molecules in the sample are proteins. The biuret method utilises a spectrophotometer to measure a colorimetric reaction between copper ions and the amide groups in proteins resulting in a measured protein concentration. The biuret method is considered to be the ‘gold standard’ for measurement of total protein in serum and plasma however, samples often have to be submitted to an external laboratory.

Precision of the refractometer

Measurement of TPPref relies on visual assessment by the operator which may lead to significant intra-observer variability and inter-observer variability. One study found a low covariance (0.9%) when the same operator read total protein 10 times on the same sample of cow serum, however, this study was not measuring total protein in plasma, did not test intra-observer variability over a range of TPP concentrations and did not test inter-observer variability. Therefore, precision of the refractometer for measuring TPP still needs to be fully evaluated before comparisons can be made to TPPbiuret.

One variable that may affect accuracy of refractometry is the presence of macroscopic abnormalities of the sample, such as lipaemia and haemolysis. In the same study mentioned above, inclusion of plasma samples that had macroscopic abnormalities decreased the correlation coefficient between TPPbiuret and TPPref to r=0.632. Similarly, refractometry can over-estimate TPP when other large molecules, such as artificial colloid molecules, are present in the sample.

Refractometry vs Biuret method

One previous study using dog plasma assessed the relationship between TPPbiuret and TPPref, and found good correlation when there were no macroscopic abnormalities (r = 0.84). This study also showed a consistent difference between the two measurements, however, most samples centred around the reference interval for TPP in healthy dogs. Therefore, this study did not adequately challenge the ‘agreement’ of the two tests. To determine agreement between two methods that measure the same quantity, a Bland-Altman analysis should be
performed with a sample range that represents the limits of the tests. Currently, no studies have assessed the agreement between these two methods of measuring TPP in dogs.

**Artificial colloids and colloid osmotic pressure**

A similar study assessing the effect of artificial colloid fluids (6% Dextran 70, Gentran® 70, Baxter Healthcare, US and 6% HES 130/0.4 Hespan®, DuPont Pharmaceuticals, US) on TPPref and COP(12) found a significant increase in COP without a significant change in TPPref when 6% Dextran 70 and 6% HES 130/0.4 were added to human serum albumin at a range of dilutions. The use of human serum albumin is a limitation when trying to extrapolate these results to canine patients due to species differences in albumin: globulin ratio. This study assessed the relationship between TPPref and COP when artificial colloid fluids were added to human serum. However, the relationship between TPPbiuret and COP has not been evaluated when artificial colloid fluids are added to canine plasma.

Another study evaluating the use of 6% HES 130/0.4 (Hespan®, DuPont Pharmaceuticals, US) in a small population of hypalbuminaemia dogs found the mean COP after one dose of 6% HES 130/0.4 to be significantly higher post treatment.(14) There was no correlation between dose of artificial colloid fluid and change in COP or resolution of interstitial oedema.(14)

Most general practices in Australia have access to one or more types of colloid fluids and use them regularly. Animals that receive artificial colloid fluids are also those that require regular TPP monitoring. However, administration of artificial colloid fluids may affect in-house monitoring of TPPref and produce erroneous results, possibly leading to inappropriate treatment decisions. Additionally, the predictable and positive relationship between COP and TPPbiuret is likely to be lost once artificial colloid fluids are used. It is anticipated that as COP increases with addition of artificial colloid molecules, that TPP will become diluted and, hence, decrease. This relationship of increasing COP in the face of a decreasing TPPbiuret concentration has not been described in the veterinary literature.

**Study aim and design**

The overall aim of this in vitro study was to assess the effect of two different artificial colloid fluids, 6% HES 130/0.4 (Volume®, Fresenius-Kabi, Australia) and 4% succinylated gelatine solution (Gelfusine®, B. Braun, Germany) on the refractometric and biuret measurement of total plasma protein (TPP), and the relationship between TPP and colloid osmotic pressure (COP). The accuracy and precision of the hand-held refractometer (Goldberg TS meter, Reichert, USA) was established by assessing the intra-observer and inter-observer repeatability of measuring TPP over a range of protein concentrations created by diluting plasma with saline. The agreement between refractometry and biuret method (Randox Daytona, UK) for measuring total plasma protein was evaluated when 6% HES 130/0.4 and 4% succinylated gelatine solution was added to plasma in vitro at a range of dilutions. Finally, the relationship between TPPbiuret and COP (4420 Colloid Osmometer, Wescor, USA) of plasma samples diluted with saline was compared to the relationship between TPPbiuret and COP once artificial colloid fluids were added to plasma in vitro, at a range of dilutions.

**Study findings and clinical relevance**

The hand-held refractometer showed excellent intra-observer and inter-observer repeatability and the equipment can be used by multiple operators in a practice with similar results from the same sample expected. TPPbiuret and TPPref did not have perfect agreement and the difference between the two measured variables was random, with TPPref measurements being on average 0.5 g/L (bias) higher than TPPbiuret measurements with 95% limits of agreement of -4.0 to 5.0. It is important for clinicians to be aware of this difference and to interpret TPP readings that are gained by two different techniques appropriately.

The addition of 6% HES 130/0.4 to plasma resulted in disagreement between TPPref (voluven) and TPPbiuret (saline) measurements and on average, were 13.7 g/L (bias) higher than TPPbiuret (saline) with 95% limits of agreement 0.3-27.0. The disagreement was the most at lower average TPP concentrations. In patients that have received HES, measurements of TPP via refractometry are inaccurate, systematically biased and will always read above the true protein concentration, being more biased as more HES is administered.

The addition of 6% HES 130/0.4 to plasma resulted in disagreement between TPPbiuret (voluven) and TPPbiuret (saline) although not as pronounced as the refractometry result. The TPPbiuret (voluven) was on average 0.7 g/L (bias) higher than the TPPbiuret (saline) with 95% limits of agreement of -3.6 to 5.0, with the disagreement
appearing random. The biuret measurement of a plasma sample diluted with HES will result in a concentration that could be as much as 5.0 higher or -3.6 lower than the true value and the variation is unpredictable (i.e. at random), making interpretation of TPP$_{\text{biuret}}$(voluven) difficult.

The addition of 4% succinylated gelatine solution to plasma resulted in disagreement between both TPP$_{\text{ref}}$(gelofusine) and TPP$_{\text{biuret}}$(gelofusine) versus TPP$_{\text{biuret}}$(saline). The disagreement was the most at lower concentrations of total protein. The refractometry measurements were on average 11.6 g/L higher (bias) with 95% limits of agreement 0.3-23.0 and the TPP$_{\text{biuret}}$(gelofusine) measurements were on average 9.9 g/L higher (bias) with 95% limits of agreement -0.2-19.9. These findings imply that in patients that have received gelofusine, measurements of TPP via both refractometry and biuret method are inaccurate and biased as gelofusine interferes with both methods of measurement.

Finally, the addition of 6% HES 130/0.4 and 4% succinylated gelatine solution to plasma increased COP and decreased TPP$_{\text{biuret}}$ measurements. This inverse relationship is clinically relevant as TPP measured by the biuret method will no longer be an indicator of COP once artificial colloid fluids are administered to a patient.
References