Comparison of four methods for determination of total protein concentrations in pleural and peritoneal fluid from dogs

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Objective—To compare 4 techniques for determination of total protein concentrations in peritoneal and pleural effusions from dogs.

Sample Population—23 peritoneal and 12 pleural fluid samples from 35 dogs with various abnormalities.

Procedure—Samples were collected into tubes containing EDTA, centrifuged, and stored at –20 C until total protein concentrations were assessed. Protein concentration in each sample was determined by use of urine test strips, refractometer, and Bradford and biuret techniques. Accuracy of each method was determined, using dilutions of human control sera.

Results—There was good correlation among results of all quantitative procedures. Results of the biuret technique were more accurate than results of the Bradford assay. Refractometry underestimated protein concentration in samples with < 20 g of protein/L. Results of urine test strips correctly classified effusion samples into 2 groups on the basis of total protein concentrations: less than or greater than 20 g/L.

Conclusions and Clinical Relevance—Results of any of these 4 techniques can be used to rapidly and efficiently differentiate peritoneal and pleural fluid from dogs into transudates and exudates on the basis of total protein concentration less than or greater than 20 g/L, respectively. (Am J Vet Res 2001;62:294-296)

Regardless of localization, classification of serous effusions as transudates or exudates is the first step in the diagnosis of the cause of abnormal fluid collections. The formation of transudates is attributable to systemic factors that result in development of a diluted clear fluid, whereas the formation of exudates is attributable to inflammation or neoplasia resulting in a fluid that may macroscopically resemble plasma.

In human medicine, the most frequently used criteria for differentiating transudates from exudates are total protein concentration and nucleated cell count. Protein concentration can be measured with a refractometer or by use of colorimetric procedures (eg, biuret and Bradford techniques). To our knowledge, these methods have not been validated for analysis of effusions in dogs. In addition, rapid measurement techniques, such as those incorporating urine test strips, have not been evaluated for this use. The purpose of the study reported here was to compare 4 methods for determination of total protein concentrations in effusions from dogs.

Materials and Methods

Samples—Twelve pleural and 23 peritoneal effusion samples were collected from 35 dogs with various diseases. Samples were collected into tubes containing EDTA, centrifuged for 3 minutes at 2,000 X g, and stored frozen at –20 C in microfuge tubes until analyzed. All samples were clear, some were slightly red, and none were lactescent or icteric.

Determination of total protein concentration—Total protein concentration in each sample was measured by use of urine test strips, a clinical refractometer, the biuret technique, and the Bradford assay. To determine total protein concentration by use of urine test strips, 10 μL of each sample was pipetted onto the protein reagent patch and allowed to incubate for 60 seconds. Color was evaluated by the same person according to the manufacturer’s color chart. Samples were assigned to 1 of the 4 following groups on the basis of total protein concentration: negative, 0.3 to 1 g/L, 1 to 3 g/L, 3 to 20 g/L, and > 20 g/L.

Before determining total protein concentrations with a clinical refractometer, the 1,000 density reading was validated with distilled water. Twenty microliters of each sample was then pipetted on the prism, and the protein concentration was read on the serum protein scale. Limits of quantification were determined, using sequential dilutions of canine plasma samples. Results indicated that there was an excellent correlation (r = 0.999) between expected and measured protein concentrations at concentrations as low as 4 g/L. At protein concentrations < 4 g/L, the difference between measured and expected results could be as much as 2 g/L. The coefficient of variation was 2%, as determined by 10 repeated measurements of a standard containing 4.5 g of bovine albumin/L. A limit of quantification was not indicated by the manufacturer.

The final method used to determine total protein concentra-
Total protein concentrations in all effusion samples ranged from 0 to >60 g/L. Results of the 2 colorimetric procedures (ie, biuret and Bradford techniques) were strongly correlated (r = 0.966, P < 0.001; Fig 1). However, biuret results were significantly (P < 0.001) greater than Bradford results (linear regression equation: Bradford result = 0.94 × biuret result − 0.75). Accuracy of the biuret technique was good; results for human control sera were within 0.3 g/L of target values. Results of the Bradford assay underestimated total protein concentration by 1 to 1.5 g/L in dilutions of control sera containing 6.9 and 5.9 g of protein/L.

Total protein concentrations in effusion samples determined by use of refractometry correlated well with biuret and Bradford results (r = 0.982 and 0.964, respectively). However, refractometry did not detect protein in effusion samples with 4 to 6 g of protein/L as determined by the Bradford or biuret technique. Moreover, refractometry results underestimated protein concentrations in human control sera containing less than 20 to 25 g of protein/L.

Semiquantitative evaluation of total protein concentrations in effusion samples, using urine test strips, was difficult because there were only slight color differences among samples with different grades (ie, negative, 0.3 to 1 g/L, 1 to 3 g/L, 3 to 20 g/L, and >20 g/L). Nonetheless, we did detect a good relationship between test strip results and results of the other 3 methods (Fig 2). Moreover, assignment of effusion samples to 4 classes on the basis of results of the test strips was good. The only discrepancies were with refractometry results, which underestimated low protein concentrations. Classification of effusion samples into 2 groups on the basis of total protein concentrations less than or greater than 20 g/L was the same, regardless of the technique used to determine protein concentration.

Discussion

Transudates and exudates are usually characterized by a protein concentration less than or greater than 25 g/L, respectively. No specific procedures have been reported for analysis of protein concentrations in effusions of animals. However, many textbooks and articles mention the use of refractometry.

In humans, transudates and exudates may contain enough protein to allow measurement of total protein concentration by use of the biuret method. This is probably not true for samples with low concentrations of proteins, as the limit of quantification for the biuret method is 1 to 15 mg of protein in the sample aliquot measured. This translates to approximately 2 to 10 g of protein/L. Refractometry is likely to be inaccurate for measurement of protein concentrations in human effusions that contain <35 g of protein/L. Coomassie blue, which is used in the Bradford assay, is commonly used to determine the amount of protein present. However, it is not as sensitive as the biuret method and may not be able to detect protein concentrations <1 g/L.
used to measure low concentrations of proteins. Coomassie blue reacts more intensely with albumin than globulins, which could make it a preferred reagent because transudates contain mainly albumin.6

Urine test strips allow the semiquantitative determination of protein concentrations; the limit of detection may be as low as 0.3 g of protein/L. However, use of these test strips cannot differentiate samples with protein concentrations > 20 g/L. The test strips also react more intensely with albumin than globulins. Possible errors of interpretation resulting from observation of the green color change on the test strips can be avoided by use of automated readers, although such readers are expensive.

Our results indicate that the 4 techniques we evaluated can be satisfactorily used to classify effusion samples from dogs into 2 groups on the basis of protein concentrations less than or greater than 20 g/L, even though moderate differences in precision and accuracy were observed. If a quantitative measurement of protein concentrations is needed, we recommend use of the biuret technique for samples with high protein concentrations (≥ 5 g/L); the biuret technique was more accurate than the Bradford assay for samples with high protein concentrations. On the other hand, for samples with concentrations < 5 g/L, only the Bradford assay or similar techniques can correctly quantify proteins.7 Refractometry may be useful for evaluation of effusions from dogs, although refractometry underestimated protein concentration in samples with < 20 g of protein/L. In addition, in all effusion samples with > 20 g of protein/L, the difference between biuret and refractometer results was low. If a threshold of 20 g/L were considered adequate to differentiate between exudates and transudates, all techniques, including those incorporating urine test strips, would provide the same results without misclassifications.

References