The Veterinary Journal 194 (2012) 332–337

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Evaluation of an automated assay based on monoclonal anti-human serum amyloid A (SAA) antibodies for measurement of canine, feline, and equine SAA ☆

M. Christensen [a,*], S. Jacobsen [b], T. Ichiyanagi [c], M. Kjelgaard-Hansen [a]

[a] Department of Small Animal Clinical Sciences, University of Copenhagen, Denmark
[b] Department of Large Animal Sciences, University of Copenhagen, Denmark
[c] EIKEN Chemical Company, Tokyo, Japan

ARTICLE INFO

Article history:
Accepted 7 May 2012

Keywords:
Immunoassay
Validation
Acute phase protein
Companion animals
Amyloid

ABSTRACT

Major acute phase proteins (APPs) have proven diagnostically useful in dogs, cats and horses with routine use facilitated by commercially available automated heterologous assays. An automated assay applicable across all three species would highly facilitate further dissemination of routine use, and the aim of this study was to validate an automated latex agglutination turbidimetric immunoassay based on monoclonal anti-human serum amyloid A (SAA) antibodies for measurement of canine, feline and equine SAA. Serum samples from 60 dogs, 40 cats and 40 horses were included. Intra- and inter-assay imprecision, linearity and detection limit (DL) were determined to assess analytical performance. To assess clinical performance, equine and feline SAA measurements were compared with parallel measurements using a previously validated automated SAA assay in a method comparison setting, and by assessing overlap performance of canine SAA in healthy dogs and diseased dogs with and without systemic inflammation.

Intra- and inter-assay CVs ranged between 1.9–4.6% and between 3.0–14.5%, respectively. Acceptable linearity within a clinically relevant range of SAA concentrations was observed for all three species. The DL was 1.06 mg/L. Method comparison revealed acceptable agreement of the two assays measuring feline and equine SAA, and the overlap performance of canine SAA was acceptable. The tested assay measured SAA in canine, feline and equine serum with analytical and overlap performance acceptable for clinical purposes so improving practical aspects of clinical APP application. The monoclonal nature of the antibodies suggests strong, long-term inter-batch performance stability.

Introduction

Major acute phase proteins (APPs) have proven to be diagnostically useful as routine inflammatory markers in major companion animal species including dogs, cats, and horses (Sasaki et al., 2003; Jacobsen and Andersen, 2007; Nakamura et al., 2008). Automated human turbidimetric immunoassays (TIA) have previously been validated for measurements of feline and equine serum amyloid A (SAA) (Hansen et al., 2006; Jacobsen et al., 2006a) and canine C-reactive protein (CRP) (Kjelgaard-Hansen et al., 2003; Kjelgaard-Hansen, 2010; Klenner et al., 2010), facilitating dissemination of the routine applicability of APPs (Kjelgaard-Hansen and Jacobsen, 2011). However, even though their diagnostic potential has been known for decades, routine measurement of APPs is still not as widely applied as expected (Eckersall, 2004).

Development of an automated assay applicable for measurements of major APPs across all three species (canine, feline and equine) would markedly improve the practical aspects and dissemination of measuring major APPs for routine diagnostic purposes. SAA is an obvious target for a multi-species assay as it is a major APP in all species of interest in veterinary medicine (Kjelgaard-Hansen and Jacobsen, 2011). Moreover, the diagnostic potential of SAA measurement in feline and equine medicine is well established (Sasaki et al., 2003; Jacobsen and Andersen, 2007) and several studies have shown that canine SAA also can be diagnostically useful as a routine inflammatory marker most likely comparable to the diagnostic capacity of other canine APPs (Chikamune et al., 1998; Dabrowski et al., 2007, 2009). For diagnostic laboratories investing in APP assays, the presence of a single assay for several animal species would be an obvious advantage (Kjelgaard-Hansen and Jacobsen, 2011). Furthermore, if the assay was based on monoclonal antibodies, stable long-term and inter-batch performance would be possible (Kjelgaard-Hansen, 2010).

The aim of the present study was to evaluate the analytical performance of an automated latex agglutination turbidimetric immunoassay (LAT) based on monoclonal anti-human SAA

☆ Part of this study was presented at the 13th Conference of the European Society of Veterinary Clinical Pathology, Dublin, 3 September 2011.

* Corresponding author. Tel.: +45 35332952.
E-mail address: mc@life.ku.dk (M. Christensen).

1090-0233/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2012.05.007

antibodies to measure SAA in dogs, cats, and horses. The evaluation included an assessment of imprecision, inaccuracy and detection limit (DL), as well as a comparison with an established method for diagnostic measurements of feline and equine SAA and an evaluation of the overlap performance of canine SAA.

Materials and methods

Assay

An LAT based on monoclonal anti-human SAA antibodies was evaluated in the study. The assay was developed in collaboration with EIKEN Chemical Company for measurements of canine, feline, and equine SAA. Data demonstrating the specificity of the monoclonal antibodies in the detection of SAA are provided in Appendix A (Supplementary material). The measurements with the LAT and the commercial available human SAA TIA (Hansen et al., 2006; Jacobsen et al., 2006a) were performed using an automated clinical chemistry analyser (ADVIA 1800, Siemens). The LAT was calibrated using heterologous calibration material, meaning that the concentrations measured in mg/L were in human equivalents of SAA, rather than exact concentrations of species-specific SAA.

Samples

Serum samples from 60 dogs, 40 cats and 40 horses obtained for diagnostic purposes were included in the study. All samples were taken from client-owned animals. The study was approved by the local ethical committee. All animals underwent clinical examination during the period January 2010 to May 2011, and blood samples were analysed at the Central Laboratory, Department of Small Animal Clinical Sciences, University of Copenhagen, Denmark. Remaining serum was stored in plastic vials at −20 °C until analysis. Samples were only thawed when needed for analysis in order to limit freeze–thaw cycles.

Assay characteristics

The intra- and inter-assay variations were determined as the coefficient of variation (CV) based on replicate measurements of SAA in serum pools within the same analytical run (same day) (n = 8–16) and across analytical runs (different days) (n = 6–8), respectively. Pools containing intermediate concentrations of SAA (40–45 mg/L for horse and cat and 70–75 mg/L for dogs, respectively) and pools with high concentrations of SAA (>900 mg/L for canine SAA, >170 mg/L for feline SAA, and >1500 mg/L for equine SAA) were used. The DL was calculated from replicate determinations of blanks (distilled water) (n = 8).

Inaccuracy was investigated by linearity under dilution. Pooled samples with very high concentrations of canine and feline SAA were initially diluted 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% using distilled water; subsequently, in order to get a more detailed picture of the inaccuracy at high and low concentrations, additional dilutions were made (5%, 15% and 95%). Because extremely high concentrations of SAA are occasionally observed in equine serum (Jacobsen et al., 2006b), an even more detailed spectrum of dilutions of such an extreme sample were made for this particular species, namely, 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% and 100%. This was done to test for possible pro-zone effect having a clinical impact. Expected concentrations of SAA for each dilution were calculated from the observed concentrations in undiluted canine and feline serum and in 25% dilution of the equine serum, respectively, and the linearity was investigated visually and by linear regression.

A method comparison for measurements of feline and equine SAA was performed comparing results obtained by the LAT to measurements of SAA using a commercial available TIA validated for SAA measurements in cats and horses (Hansen et al., 2006; Jacobsen et al., 2006a). Parallel measurements of SAA in 40 individual equine serum samples and 40 individual feline serum samples were used for the method comparisons (Jensen and Kjelgaard-Hansen, 2006). The serum samples were chosen in order to cover the full spectrum of SAA concentrations most frequently expected in clinical settings. The LAT was considered reliable for diagnostic measurements of feline and equine SAA if the performance could be shown to be comparable to the reference method already used for routine diagnostic measurements of SAA (Jacobsen and Andersen, 2007).

Overlap performance

SAA is a major positive APP in dogs (Cerón et al., 2005). If the LAT should be considered reliable for diagnostic assessment of the acute phase response, the overlap performance of SAA measured by the LAT should be acceptable and able to discriminate dogs with inflammatory disease from healthy dogs and dogs without inflammatory disease (Kjelgaard-Hansen et al., 2003; Nakamura et al., 2008; Yuki et al., 2010; Hagman, 2011). Based on the final clinical diagnoses, 60 dogs were retrospectively assigned to three groups: (1) clinically healthy dogs (n = 20); (2) dogs with systemic inflammation (n = 20), and (3) diseased dogs without systemic inflammation (n = 20). For all dogs basic hematologic and clinical chemical profiles were analysed (Jensen et al., 2001). Additional diagnostic tests for each dog were conducted at the discretion of the individual clinician (e.g. radiography, ultrasonography, MRI, CSF, endocrine testing, cytology or histopathology).

Clinically healthy dogs had unremarkable findings on physical and clinical pathological examinations (n = 20). Diseased dogs without systemic inflammation were diagnosed with idiopathic epilepsy (n = 7), syringomyelia (n = 2), cardiac disorders (myxomatous mitral valve disease or dilated cardiomyopathy) (n = 3), arthrosis (n = 2) or other disorders without any signs of systemic inflammation (n = 6). Dogs classified with systemic inflammation were hospitalized because of trauma (n = 4), pyometra (n = 2), severe meningitis (n = 3), acute gastroenteritis (n = 4), acute symptoms of chronic gastrointestinal disorders (n = 2) or other disorders with a systemic inflammatory response (n = 5).

A comparable spectrum of ages, sexes, and breeds were included in each group.

Statistics

Intra- and inter-assay CVs were calculated using routine descriptive statistical procedures (Büttner et al., 1980). Imprecision performance was deemed acceptable if comparable to previously validated automated assays measuring major APPs, which have demonstrated sufficient analytical performance for clinical applicability (Kjelgaard-Hansen et al., 2003; Hansen et al., 2006; Jacobsen et al., 2006a). Investigation of linearity under dilution was accomplished by Deeming regression analysis. Linearity performance was deemed acceptable if slope and Y-intercept did not deviate from 1 and 0, respectively.

The method comparison was performed by Deeming regression, and the Pearson's correlation coefficients were calculated. Agreement between methods was assessed by deviations from line of agreement (Y = X). Pearson's correlation coefficients close to 1 indicated positive correlation.

Runs test was performed in all types of regression analyses to determine whether data deviated significantly from the applied linear model. Overlap performance was assessed by comparison of SAA concentrations among groups of dogs using Dunn's multiple comparison test and visual assessment of scatter plots. Significant discrimination between the group of dogs with systemic inflammation and other groups were set as criteria for acceptability. Significance was set at $P < 0.05$.

Results

Assay characteristics

Intra- and inter-assay CVs ranged from 1.9% to 4.6% and from 3.0% to 14.5%, respectively, in measurements of high and intermediate concentrations of SAA (Table 1). When investigating linearity under dilution, a deviation from linearity was observed in the measurements of high concentrations of SAA limiting the working range of the assay for all three species (Fig. 1A–C). Introduction

Table 1
Intra- and inter-assay coefficients of variation (CV) for canine, feline, and equine serum amyloid A (SAA) measured by a latex agglutination immunoassay based on monoclonal anti-human SAA antibodies. SD, standard deviation.

	Canine SAA	Feline SAA	Equine SAA
Intra-assay CV Intermediate concentration			
Range (mg/L)	[67.7; 75]	[39.9; 46.2]	[42.8; 45.2]
Mean (mg/L)	72.6	44.1	43.5
SD	3.0	2.0	0.9
CV (%)	4.1	4.6	2.1
Intra-assay CV High concentration			
Range (mg/L)	[909.2; 973.6]	[170.5; 188.9]	[1491; 1668]
Mean (mg/L)	931.6	177.6	1612
SD	17.5	7.1	52.1
CV (%)	1.9	4.0	3.2
Inter-assay CV Intermediate concentration			
Range (mg/L)	[55.2; 77.5]	[39.4; 46.1]	[34.3; 46.1]
Mean (mg/L)	68.6	42.7	41.3
SD	8.6	2.9	4.8
CV (%)	12.5%	6.7%	11.6
Inter-assay CV High concentration			
Range (mg/L)	[687.5; 946.0]	[130.5; 188.9]	[1491; 1668]
Mean (mg/L)	801.5	160.8	1543
SD	77.6	23.3	46.0
CV (%)	9.7	14.5	3.0