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ORIGINAL ARTICLE

Comparison of feline serum amyloid A (SAA) measurements assessed by a point-of-care test analyzer to a validated immunoturbidimetric method

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Abstract Serum amyloid A (SAA) is one of the most important acute phase proteins in cats and increases rapidly and significantly after an inflammatory stimulus. The objective of this study was to compare measurements of SAA concentration in feline blood specimens using the point-of-care Eurolyser Solo Analyzer (SOLO) with results of a validated immunoturbidimetric test. This prospective study was conducted between March 2014 and June 2015 at a university teaching hospital. Blood specimens were collected from a total of 61 cats including 45 client-owned cats with inflammatory diseases presenting to the emergency and critical care service and 16 client-owned healthy cats. Before SAA measurement, the blood was centrifuged and stored at − 80 °C (− 112 °F). The Eiken assay immunoturbidimetric method (EKITM) which uses latex sensitized anti-human SAA antibodies served as reference SAA test. Serum amyloid A measurements with the EKITM were performed in a reference laboratory. Serum amyloid A measurements with the SOLO were performed in an emergency laboratory. Thirty-six and 18 results were out of the analytical range with the EKITM and the SOLO, respectively. The correlation between the two methods was high ($r = 0.87$) and there was an intense mostly proportional bias, with results being approximately 2 times lower with the SOLO than the reference test. This study showed that the SOLO reliably and rapidly measures SAA in feline blood specimens; however, a "de novo" reference interval should be determined for proper interpretation of the results.

Keywords Inflammation · Serum amyloid A · Acute phase protein · Feline · Point-of-care test

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Introduction

Inflammatory processes are common in cats admitted to an emergency and critical care service but can be difficult to identify because of the diversity of the underlying diseases and the inconsistency of specific clinical changes.

Findings on physical examination, such as a change in body temperature, are neither consistent nor sensitive markers of inflammation (Brady et al. 2000, Ruthrauff et al. 2009, Segev et al. 2006, Sergeeff et al. 2004). Clinical pathology abnormalities may be helpful to identify patients with inflammatory diseases. Analysis of total WBC count and blood smear evaluation to assess WBC differential and WBC toxicity have been used to identify patients with inflammatory diseases; however, abnormalities are not consistent and their identification can be time consuming and requires expertise (Segev et al. 2006). Other biological abnormalities, such as changes in albumin to globulin ratio, may be suggestive of an inflammatory process and can be measured rapidly but lack sensitivity and specificity (Ruthrauff et al. 2009, Segev et al. 2006, Sergeeff et al. 2004).

The acute phase response, a reaction that occurs after any tissue injury, is part of the innate host defense system (Ceron et al. 2005). This response can be initiated by various causes (e.g., infection, trauma, severe inflammation, or immunologic reactions) and results in systemic effects such as fever and

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leukocytosis as well as changes in the concentration of plasma proteins called acute phase proteins (APPs). Some of these APPs decrease in concentration (negative APPs) and others increase in concentration (positive APPs). The plasma concentration of positive APPs increases rapidly and significantly after an inflammatory stimulus, making them sensitive biomarkers of systemic inflammation (Ceron 2008). In cats, SAA is considered one of the most important positive APPs (Kajikawa et al. 1999). Its concentration increases by 10- to 100- fold within 24 to 48 h of an inflammatory stimulus (Ceron et al. 2005, Kajikawa et al. 1996, Kajikawa et al. 1999, Sasaki et al. 2003). The SAA concentration decreases rapidly when inflammation subsides, making it a useful parameter to monitor the evolution of inflammation (Kajikawa et al. 1999). In a recent retrospective study, SAA has been proven to be a more sensitive inflammatory marker than WBC count and an independent prognostic factor in cats suffering from various diseases (Tamamoto et al. 2013).

The usefulness of SAA in the emergency setting has been limited by the lack of a point-of-care test (POCT) and the need to refer specimens to specialized laboratories. Recently, a POCT to measure feline SAA has been commercialized, but to our knowledge has not yet been evaluated.[1] The objective of this study was to compare SAA concentration measured in feline blood specimens using the SOLO POCT and a validated laboratory method (EKITM).[1,2] Our hypothesis was that results obtained by the POCT are comparable to those of the EKITM.

Materials and methods

Animals

Client-owned cats presented to the emergency and critical care service of the university teaching hospital between March 2014 and June 2015 with presumptive inflammatory diseases were included in the study. Informed client consent was obtained prior to study enrollment. Ethic committee agreement was not necessary because the analyses were performed for clinical patient management.

Data collection

Patients were included in the inflammatory diseases group based on clinical criteria and/or laboratory test results. Clinical criteria were: (1) a clinical diagnosis of a systemic inflammatory response syndrome as described previously (Brady et al. 2000) or (2) the identification of an inflammatory disease process on clinical evaluation (e.g., abscess, bite wound, trauma, pyothorax, peritonitis, pancreatitis, neoplasia, viral disease like feline infectious peritonitis or feline parvovirosis…). Laboratory criteria for inclusion were based on laboratory reference intervals and included one or more of the following: (1) neutrophilic leukocytosis or neutropenia with neutrophils $\geq 11.5 \times 10^9$/L or $\leq 1.18 \times 10^9$/L, respectively, (2) left shift with band cells $\geq 0.3 \times 10^9$/L, or (3) marked signs of neutrophil toxicity: grade ≥2 according to Segev et al. (2006). A group of 16 clinically healthy cats that had preanesthetic complete blood count and biochemistry profile performed before castration or ovariohysterectomy served as control.

Blood specimens were collected from unsedated sick cats and from sedated controls (diazepam and ketamine). For SAA measurements, blood was collected from the jugular vein into a dry vacuum tube and maintained for 30 min at room temperature.[3] Specimens were then centrifuged for 10 min at 2700 g and separated into two 500 μL aliquots of serum which were stored at − 80 °C (− 112 °F) for up to 18 months before measurement of SAA concentration.[4] There were no hemolyzed, lipemic, or icteric specimens in the control group and samples were not controlled for hemolysis, lipemia, or icterus in cats with inflammatory diseases. An immunoturbidimetric method using latex sensitized anti-human SAA antibodies, previously validated for use in cats, served as the reference SAA test (Hansen et al. 2006).[5] A calibration curve was created using the human SAA calibrator from the same manufacturer.[2] Specimens were analyzed on a fully automated clinical chemistry/immunoassay analyzer.[6] Two control specimens were analyzed prior to analyzing the study specimens; one was a commercial control based on human sera (11 mg/L),[2] and the other a feline control prepared in the laboratory from sera of sick cats (113 mg/L). The intra-assay coefficients of variation (CV) for feline specimens were determined by analyzing control solutions with a SAA concentration of 11 and 113 mg/L. The CV were 9.7 and 1.5%, respectively. The analytical range of the reference method used in the current study was 10–140 mg/L (data not shown), and specimens with SAA concentrations > 140 mg/L were manually diluted 1:3 with saline and re-analyzed, extending the upper limit of the analytical range to 420 mg/L.

Point-of-care test analyses were performed using the SOLO and manufacturer's reagents.[1] The SOLO uses a latex agglutination test and an optical measurement. Latex particles sensitized with SAA antibodies agglutinate with feline SAA which causes a change in turbidity detected by the analyzer. According to the manufacturer, the assay has a linear range of 10–150 mg/L. Although recommended by the manufacturer

[1] Solo, Eurolyser Diagnostica GmbH, Salzburg, Austria
[2] Eiken assay LZ test SAA, Eiken Chemical Co, Tokyo, Japan, lot n°46,007
[3] Venosafe® 3 mL, Terumo, Europe N.V, Leuven, Belgium
[4] SIGMA 3 K10® Laborzentrifugen, Bioblock Scientific
[5] VT 1700 SAA Control, Solo, Eurolyser Diagnostica GmbH, Austria - mean and range of acceptability: 101 mg / L and 66–136 mg / L respectively
[6] Abbott Architect c4000, Abbott Park Road, Libertyville Township, IL, USA

specimens in which SAA concentration was > 150 mg/L were not diluted because we wanted to stay as true as possible to the routine emergency setting in which the clinician may not have the time or the financial resources to dilute and re-analyze the sample. Between-run and within-run imprecision of the SOLO was only evaluated with a pool of feline sera with a mean SAA concentration of 113 mg/L. The evaluation was performed on five consecutive days, as recommended by the National Committee on Clinical Laboratory Standards (Carey et al. 2005). Control analyses were performed before each series of measurements with the manufacturer's control solution and bias was estimated as Bias (%) = (target – measured mean)/target * 100.[5] The total observed error of the SOLO was calculated using the formula TEobs = 2CV + Bias (Harr et al. 2013). Analyses of the specimens were performed in batches of 5 to 10 with the SOLO by the same person (VL), and in a single run with the EKITM.[1,2]

Statistical analysis

Paired results obtained by the two methods were compared as recommended by Spearman's correlation, Passing-Bablok agreement test and difference diagram (Jensen and Kjelgaard-Hansen 2006).

As some results were outside the analytical limits of the methods, comparisons were carried out in three steps: (1) considering only the results which were within the analytical range of both methods ($n = 19$); (2) results within the analytical range of both methods plus SOLO results > 150 mg/L ($n = 25$); (3) all results while using a value of 10 mg/L for results lower and 420 mg/L for results higher than the limits of the analytical range ($n = 61$). Calculations were performed with an Excel spreadsheet and Analyse-It.[7]

Results

Total imprecision of the SOLO with the feline pool of serum was 9.2% at a mean SAA concentration of 113 mg/L. Mean bias with the manufacturer's control solution was 6 mg/L, i.e., 6.0%. Thus, the total observed error was 24.4%.

Paired results from 61 cats were available (45 from the inflammatory diseases group and 16 from the control group). Some results were out of the analytical range: 25 and 3 results were < 10 mg/L with the EKITM and SOLO, respectively, 15 results were > 150 mg/L with the SOLO, and 11 results were > 420 mg/L with the EKITM. In all cases, i.e., including or not the results outside the analytical range (see above), the correlation between the two methods was high. However, there was an intense, mostly proportional error (Table 1), with results being approximately 2 times lower with the SOLO than with the EKITM (Figs. 1. and 2).

[7] Analyse-It, Leeds, UK

Discussion

The results of this study show that measurements of feline SAA by the reference method (EKITM) and the SOLO POCT are correlated but not commutable.

The imprecision of the SOLO was similar to that of other feline SAA assays (Kajikawa et al. 1996, Hansen et al. 2006, Christensen et al. 2012). At a SAA concentration of 113 mg/L, the imprecision of the SOLO was 9.2% compared to 8.0% in a study by Hansen et al. (Hansen et al. 2006). The TEobs of 24.4%, although high, is considered acceptable for clinical use (Harr et al. 2013).

A high degree of correlation was observed between the two methods. This was expected as the EKITM and the SOLO use antibodies produced by the same manufacturer. Nevertheless, a high proportional bias and a negligible constant bias were calculated. This precludes the possibility of commutability between the two analyzers. It is thus necessary to determine a new reference interval for the SOLO according to Clinical and Laboratory Standards Institute recommendations, either by transfer of a previously determined reference interval (which to our knowledge is currently not available) or by the de novo determination of a reference interval (Horowitz et al. 2008, Geffre et al. 2009, Friedrichs et al. 2012).

Our study has several limitations: (1) Duplicate measurements of SAA were not performed in the comparison study, which probably had little influence on the comparison as the precision of the two analyzers was good; (2) The absence of a dilution study for determination of inaccuracy; (3) Specimens with SAA values > 150 mg/L using the SOLO were not diluted to obtain definitive results. The main interest of POCT analyzers such as the SOLO is the possibility to rapidly differentiate patients with inflammatory diseases from those without inflammatory diseases. We believe that the

Table 1 Comparison of feline SAA measurements by Eurolyser Solo Analyzer with the Eiken validated method ($n = 19$: results within the analytical range for both methods; $n = 25$: as above plus Eurolyser Solo Analyzer results higher than 150 mg/L; $n = 61$: all results while using a value of 10 mg/L for results lower and 420 mg/L for results higher than the limits of the analytical range; 95% confidence intervals in parentheses). (SAA: serum amyloid A)

	Spearman's R	Passing-Bablok agreement equation $SAA_{Solo} = a * SAA_{Eiken} + b$	
		a (slope)	b (intercept)
$n = 19$	0.87 (0.68–0.95)	0.38 (0.29–0.56)	24 (9–33)
$n = 25$	0.86 (0.70–0.94)	0.50 (0.38–0.60)	15 (5–34)
$n = 61$	0.91 (0.86–0.95)	0.54 (0.50–0.58)	15 (12–17)

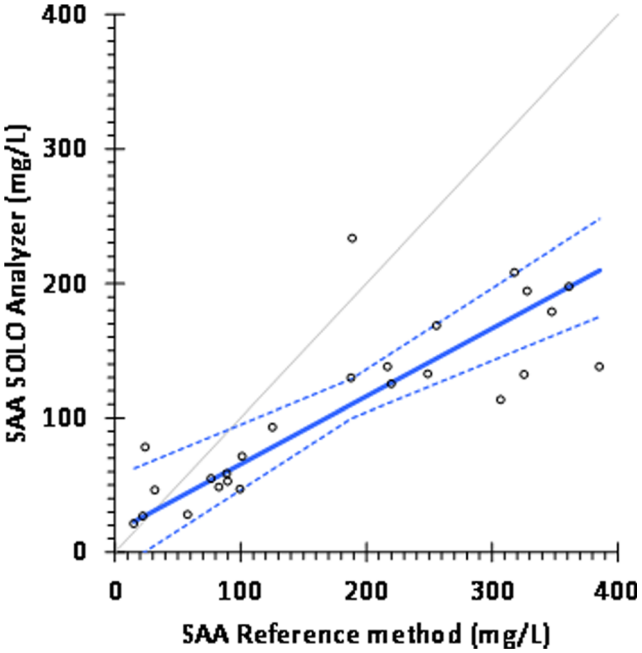

Fig. 1 Passing-Bablok agreement between results of feline SAA measurements with the Eiken validated method and the Eurolyser Solo Analyzer ($n = 25$, results within the analytical range for both methods ($n = 19$) plus Eurolyser Solo Analyzer results greater than 150 mg/L ($n = 6$); blue lines: regression curve with 95% confidence limits; gray line is equivalence). *(SAA: serum amyloid A)*

identification of an elevated value is initially of greater clinical importance than to obtain a definitive value. It is in our opinion unlikely in particular in the emergency setting that SAA

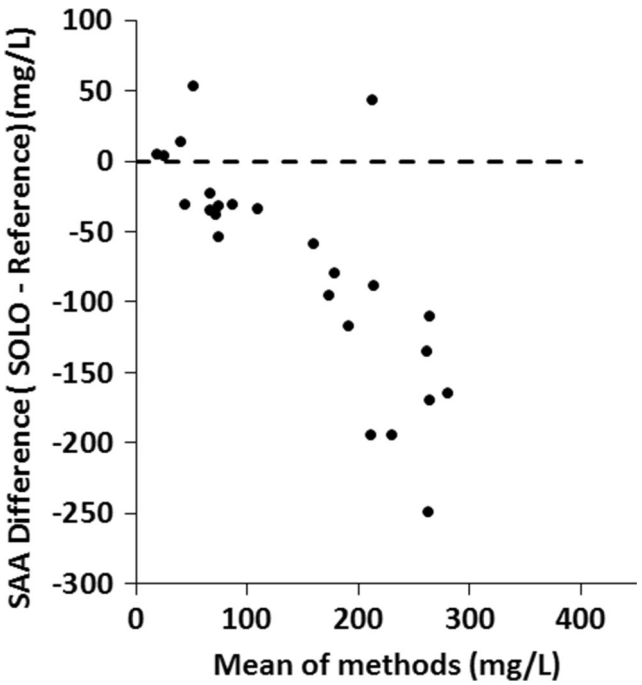

Fig. 2 Difference diagram of results of feline SAA measurements with the Eiken validated method and the Eurolyser Solo Analyzer ($n = 25$). *(SAA: serum amyloid A)*

analysis would be repeated after obtaining a positive result. Therefore, we decided to not dilute the samples with values > 150 mg/L with the SOLO method, which would have led to underestimate the value of specimens with higher concentrations. (4) The specimens were stored at − 80 °C (− 112 °F) for up to 18 months before measurement of SAA concentration. This storage may have influenced the results. Acute phase proteins are known to be very stable at − 80 °C (Ceron et al. 2005, Kann et al. 2012, Solter et al. 1991) and we therefore believe that storage had a negligible effect on the results.

Conclusion

In conclusion, the results of this study indicate that the SOLO POCT reliably and rapidly measures SAA in feline blood specimens. Even though a complete validation study would be needed to definitively assess the reliability of the SOLO analyzer, the SOLO POCT is useful to identify cats with inflammatory diseases, in particular in the emergency setting.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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