Evaluation of a Commercially Available Human Serum Amyloid A (SAA) Turbidimetric Immunoassay for Determination of Feline SAA Concentration

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ABSTRACT

Serum amyloid A (SAA) is an acute-phase protein in cats likely to be useful for diagnosing and monitoring inflammatory diseases, especially if rapid, reliable and automated assays can be made available. A commercially available automated human SAA turbidimetric immunoassay (SAA-TIA) was evaluated for determination of SAA in cats. Intra-assay and inter-assay imprecisions were in the ranges 2.1–9.9% and 7.0–12.5%, respectively, and without significant inaccuracy. Eighty-eight cats were divided into groups according to (A) the presence or absence of an acute-phase response (APR) ($n = 23$ and 65 , respectively) and (B) clinical diagnosis (clinically healthy cats, cats diagnosed with inflammatory/infectious diseases, endocrine/metabolic diseases, neoplastic diseases, and miscellaneous disorders ($n = 43, 13, 8, 4$ and 20 , respectively)). The observed SAA concentrations were, as expected, different for (A) cats with and without an APR and (B) cats with inflammatory/infectious diseases compared to other diagnostic groups, except neoplastic diseases. In conclusion, the SAA concentration in cats could be measured reliably using the commercially available TIA designed for measuring human SAA, which should facilitate implementation of the parameter for routine diagnostic purposes.

Keywords: acute-phase protein, cat, immunoassay, inflammation, serum amyloid A, test validation

Abbreviations: AGP, α_1 -acid glycoprotein; APR, acute-phase response; CI, confidence interval; CV, coefficient of variation; DSH, domestic short hair; FLUTD, feline lower urinary tract disease; SAA, serum amyloid A; SD, standard deviation; TIA, turbidimetric immunoassay

INTRODUCTION

The acute-phase response is part of the initial response to inflammatory stimuli. Following the inflammatory insult, the hepatic synthesis of a heterogeneous group of acute-phase proteins is increased (Jensen and Whitehead, 1998). Serum amyloid A (SAA) has been identified as an acute-phase marker in humans, with levels increasing 100–1000-fold in response to inflammatory stimulation (Steel and Whitehead, 1994; Gabay and Kushner, 1999; Uhlar and Whitehead, 1999). Earlier studies have concluded that SAA, α_1 -acid glycoprotein (AGP) and haptoglobin are useful indicators of the acute-phase status in cats (Kajikawa *et al.*, 1999; Sasaki *et al.*, 2003; Giordano *et al.*, 2004).

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Concerning the dynamics of SAA following an inflammatory stimulus, it has been reported that SAA increased 8 h (Kajikawa *et al.*, 1999) and 3–6 h (Sasaki *et al.*, 2003) following an inflammatory stimulus, reaching a maximum 36–48 h (Kajikawa *et al.*, 1999) and 21–24 h (Sasaki *et al.*, 2003) after initial stimulation. In comparison, AGP exhibits increased serum concentration later than SAA following an inflammatory stimulus (Steel and Whitehead, 1994), and concentrations also wane later (Kajikawa *et al.*, 1999).

The widespread use of acute-phase proteins as biomarkers for inflammatory status in human medicine has led to growing attention to their use in animal medicine (Eckersall, 2004). Significant differences in feline SAA concentration between clinically healthy cats and cats with various inflammatory diseases and disorders have already been found by direct ELISA using feline SAA-specific monoclonal antibodies (Sasaki *et al.*, 2003). However, reliable, rapid, automated and readily available assays are needed if feline SAA measurements are to be implemented for routine diagnosis and monitoring. The aim of the present study was to evaluate the validity of a commercially available automated human SAA turbidimetric immunoassay (SAA-TIA) in detecting and measuring SAA concentrations in cats, by means of assessing imprecision, inaccuracy, detection limit and overlap performance.

MATERIALS AND METHODS

Serum samples

Serum samples were obtained from 45 client-owned cats (client-owned patients) presented at the Small Animal Veterinary Teaching Hospital, The Royal Veterinary and Agricultural University of Copenhagen, Denmark, for various diagnostic, therapeutic or prophylactic measures. An additional 43 clinically healthy Maine coon cats (age and sex not registered) participating in a cardiomyopathy screening programme at the hospital were assigned to the study as a control group (healthy controls). All cats were subjected to clinical examination and standard haematological and biochemical profiles (Jensen *et al.*, 2001). Further diagnostic procedures on individual cats were conducted at the discretion of the individual clinician (e.g. radiography, cytology, histopathology, histological smears, endocrine testing).

All serum samples were obtained by centrifugation (1500g, 5 min) of blood samples after collection in plain vials containing clot activator and separation gel (Vacuette, Greiner bio-one, Kremsmuenster, Austria). Samples were stored in plastic vials at -80°C until analysis, never for longer than 4 months.

Serum amyloid A analysis

SAA concentrations were determined using a human turbidimetric immunoassay (SAA-TIA; LZ-SAA (lot no. 47007), Eiken Chemical Co., Tokyo, Japan) and analyses were performed on an automated analyser (ADVIA 1650 Chemistry System, Bayer, Newbury, UK) according to the manufacturer's recommendations. Calibration curves were created using a human SAA calibrator from the same manufacturer (Eiken Chemical Co.).

Serum α_1 -acid glycoprotein analysis

Analyses were performed using a feline-specific single radial immunodiffusion assay (Feline α_1 AG Plate, The Institute for Metabolic Ecosystem, Miyagi, Japan), following the directions supplied by the manufacturer.

Assay assessment

The SAA content was determined for all 88 cats. Three pools with high, low and medium concentration of SAA were obtained by mixing serum samples with high, low and medium SAA concentration (range, number of samples mixed): high pool 47–150.6 mg/L, $n = 3$; medium pool 21.4–33.3 mg/L, $n = 4$; low pool 1.0–3.3 mg/L, $n = 4$). The intra-assay variation was determined by measuring SAA concentration within-run on alternately high, low and medium pools seven successive times. The inter-assay variation was assessed by SAA determination of 11 between-run measurements on the same three pools. The pools were frozen in separate identical vials for the between-run analyses and only vials needed were thawed, to prevent potential variation due to repetitive freeze–thaw cycles. Linearity during dilution using physiological saline was determined to indirectly estimate inaccuracy by duplicate within-run measurements on continuous dilutions (0%, 10%, 30%, 50%, 70%, 90% and 100%) of the high SAA concentration serum pool (Jensen, 2000). Detection limit was calculated from data of 15 replicate measurements of sterile 0.9% sodium chloride (blanks).

Overlap performance

Overlap performance of the assay was assessed in two ways: (A) ability to identify cats with an acute-phase response, and (B) ability to show expected differences in SAA concentration between different groups of diseased and healthy cats. In detail: (A) The individual cat was categorized according to serum concentration of AGP, with a high concentration indicating the presence of an acute-phase response (Kajikawa *et al.*, 1999). A cut-off value of 0.65 g/L was used (based on data reported by Selting *et al.*, 2000). (B) All cats were assigned retrospectively to five different diagnostic groups according to the final diagnosis of the attending clinician, with regard to clinical, haematological, clinical biochemical and pathological findings, i.e. clinically healthy cats, cats diagnosed with inflammatory/infectious diseases, endocrine/metabolic diseases, neoplastic diseases, and miscellaneous disorders ($n = 43, 13, 8, 4$ and 20 , respectively). Cats with neoplastic diseases had tumours that were cytologically and/or histologically characterized as neoplastic. Cats with infection had a pathogen identified that could be related to the pathological findings. Cats with endocrine/metabolic disorders had a final diagnosis that could be related to hormone or metabolic malfunction/disorder. Cats with miscellaneous diseases had various diagnoses and no clinical findings to associate them with any other groups. The 43 healthy controls were assigned to the group of clinically healthy.

Overlap performance was assessed by comparing the distribution of SAA levels within the various groups in setting (A) and (B).

Statistical methods

Standard deviations, arithmetic means, medians and intra- and inter-assay coefficients of variation (CV) were assessed using standard descriptive procedures (Box *et al.*, 1978; Büttner *et al.*, 1980). Detection limit was estimated from the mean value of blanks plus three standard deviations (Jensen, 2000).

Linearity during dilution was analysed by ordinary linear regression analysis. Runs test was performed to investigate whether data followed a straight line (Jensen, 2000). The correlation of AGP and SAA was assessed by Spearman's correlation coefficient. The Mann–Whitney non-parametric test was used to investigate statistically significant differences between the SAA concentrations of cats in the groups with and without the presence of an acute-phase response according to AGP concentration (Siegel, 1998).

The Kruskal–Wallis one-way analysis was used to detect differences of medians between the diagnostic groups. On an indication of different distributions, Dunn's multiple comparison test was applied to obtain additional information regarding the deviation (Siegel, 1998). The level of significance in all tests was set at 0.05.

RESULTS

Assay characteristics

The observed intra- and inter-assay imprecisions were in the ranges 2.1–9.9% and 7.0–12.5%, respectively (Table I). Linearity under dilution was observed, with no signs of deviation from a straight line model with slope of unity and intercept of zero (Table II). Detection limit was 0.38 mg/L (mean 0.19 mg/L, SD 0.06 mg/L).

TABLE I
Observed intra- and inter-assay imprecision of feline serum amyloid A determinations by means of an automated immunoturbidimetric assay

	No. of runs	Mean value (mg/L)	SD (mg/L)	CV (%)
Intra-assay ^a	7	89.7	1.98	2.2
	7	25.8	0.53	2.1
	7	3.1	0.31	9.9
Inter-assay ^a	11	102	8.09	8.0
	11	31.1	2.22	7.0
	11	4.7	0.59	12.5

SD, standard deviation; CV, coefficient of variation

^aBased on successive measurements on alternate high, medium and low SAA concentration serum pools

TABLE II

Analytical inaccuracy of serum amyloid A measurements assessed by dilutions of a feline serum pool with a high SAA content

Regression	Y-intercept	Y-intercept 95% CI	Slope	Slope 95% CI	Runs test (<i>p</i> -value)
Linear	3.98	[−6.92; 14.89]	1.12	[0.92; 1.33]	0.41

CI, confidence interval

Clinical characteristics

Clinical characteristics, descriptive data, and serum AGP and SAA concentrations of the included cats are given in Table III. The correlation of serum AGP and SAA levels was significant ($r = 0.58$; $p < 0.0001$). Investigation of overlap performance indicated (A) that the SAA concentrations (median [range]) of the cats with (21.3 mg/L [0.3–150.6 mg/L]) and without (0.4 mg/L [0.0–60.4 mg/L]) an acute-phase response, respectively (Figure 1), were significantly different ($p < 0.0001$) and (B) that the median SAA concentrations of the various clinical groups also differed significantly ($p < 0.0001$; 0.4 mg/L [0.0–3.9 mg/L], 46.6 mg/L [1.2–150.6 mg/L], 0.5 mg/L [0.3–11.6 mg/L], 1.5 mg/L [0.2–60.4 mg/L], 0.5 mg/L [0.2–21.3 mg/L] for clinically healthy cats, cats diagnosed with inflammatory/infectious diseases, endocrine/metabolic diseases, neoplastic diseases, and miscellaneous disorders, respectively). Further comparison of the SAA concentrations in the individual groups (Figure 2) revealed that the serum SAA concentration in the group of cats with inflammation/infection was significantly higher than that of the cats belonging to the groups of clinically healthy cats ($p < 0.001$), cats with endocrine disorders ($p < 0.01$) and cats with miscellaneous disorders ($p < 0.001$). No other pair of groups differed significantly in SAA concentration.

DISCUSSION

The TIA measured feline SAA with acceptable imprecision and the investigation of linearity during dilution revealed no sign of significant inaccuracy. The observed level of SAA in clinically healthy cats (range [median] 0.0–3.9 [0.4] mg/L) was comparable to that reported by Sasaki and colleagues (2003) (0.6 ± 1.1 mg/L) but slightly lower than observations of others (20.5 ± 8.1 mg/L (DiBartola *et al.*, 1989), 16.6 ± 11.4 mg/L (Kajikawa *et al.*, 1999) and 10.2 ± 8.3 mg/L (Giordano *et al.*, 2004)). This variation between studies is most likely due to the use of different methodologies and the lack of available material for standardization of feline SAA assays. Detection limit was estimated as 0.38 mg/L, which was within the observed physiological range of SAA concentration of healthy cats. This should not impair clinical application, as the primary purpose of SAA measurements would be to identify

TABLE III

Clinical characteristics, descriptive data, serum amyloid A and α_1 -acid glycoprotein (AGP) concentrations of 88 cats grouped according to clinical diagnosis (for healthy cats only range and median given)

Clinical diagnosis	Breed	Sex (F/M)	Age (year/month)	AGP (g/L)	SAA (mg/L)
Infection/inflammation					
Keratoconjunctivitis	Siamese	F	2/10	1.5	23.6
Peridontitis/ lymphadenitis	Maine coon	M	1/0	1.3	26.9
Focal necrotic stomatitis	DSH	M	11	1.3	38.4
Dermatitis	Norwegian forest	M	1/10	1.0	3.3
Fracture	DSH	F	1/11	0.3	1.2
Severe head and pelvic trauma	DSH	M	12	2.0	150.6
Keratoconjunctivitis	Siamese	M	3/5	1.2	57.0
Acute laryngitis/pharyngitis	DSH	M	2/2	2.3	81.9
Acute pancreatitis	DSH	F	8/8	1.2	92.1
Penetrating foreign body/peritonitis	Burmese	M	0/8	1.2	46.6
Pyothorax	Maine coon	M	2/0	1.7	47.0
Deeply infected ulcer/dermatitis	DSH	F	17/1	0.9	21.4
Cystitis	DSH	M	19	1.5	50.3
Range (median)			0/8–19	0.3–2.3 (1.3)	1.2–150.6 (46.6)
Neoplasia					
Adenoma	DSH	F	6/0	0.6	0.2
Multicentric lymphosarcoma	DSH	F	14	1.1	2.5
Adenoma	DSH	F	8/8	0.3	0.4
Mastocytoma	DSH	F	10/0	0.5	60.4
Range (median)			6/0–10/0	0.3–1.1 (0.6)	0.2–60.4 (1.5)
Endocrine/metabolic disorder					
Diabetes mellitus	Siamese	F	15/7	0.3	0.7
Diabetes mellitus	DSH	F	9/0	0.4	0.9
Hyperthyroidism	DSH	F	15	0.5	11.6
Hyperthyroidism	Persian	F	12/0	0.7	0.3
Hyperthyroidism	DSH	M	16	0.4	0.3

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TABLE III
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Clinical diagnosis	Breed	Sex (F/M)	Age (year/month)	AGP (g/L)	SAA (mg/L)
Diabetes mellitus	DSH	M	9/1	0.7	0.3
Hyperthyroidism	DSH	F	N/A	0.3	0.5
Diabetes mellitus	DSH	M	9/2	0.5	0.4
Range (median)			9/0–16	0.3–0.7 (0.5)	0.3–11.6 (0.5)
Miscellaneous					
Epilepsy	Norwegian forest	F	0/11	0.7	0.4
Acute renal failure	DSH	F	15/9	0.6	3.2
Cystic kidneys	Persian	M	15	0.6	0.4
Hepatic encephalopathy	DSH	F	5/10	0.4	0.4
Vomiting	DSH	F	9/9	0.6	0.5
Hepatic steatosis	Norwegian forest	M	9/1	0.5	1.0
Hepatic cyst	DSH	F	14	0.5	0.4
Acute smoke poisoning	DSH	F	0/11	0.5	0.6
Hypertension	DSH	F	14	0.3	0.3
FLUTD	DSH	M	5/8	0.4	0.2
Anuria	DSH	M	5/3	1.6	21.3
Weight loss	DSH	F	5/1	0.4	0.3
Hepatopathy	Siamese	F	0/11	0.7	0.5
Depression	N/A	M	5	0.4	0.3
Hypoglycaemia	DSH	M	14	0.7	18.6
Chronic renal failure	Persian	F	4/8	0.4	0.4
Ileus	DSH	M	12	0.4	0.4
Pain, lumbal region	DSH	F	2/0	0.5	0.5
Neurological syndrome	DSH	M	4	0.4	0.5
FLUTD	DSH	M	7	0.5	0.6
Range (median)			0/11–15/9	0.3–1.6 (0.5)	0.2–21.3 (0.5)
Clinically healthy (n = 43)	Maine coon	N/A	N/A		
Range (median)				0.1–1.6 (0.4)	0.0–3.9 (0.4)

DSH, domestic short hair; FLUTD, feline lower urinary tract disease; N/A, not available; F, female; M, male

whether cats have an acute-phase response or not, with a clinical decision level thought to be well above 0.38 mg/L. The expected difference in serum SAA concentration were observed between (A) cats with and without an acute-phase response and (B) cats suffering from infectious/inflammatory disorders compared to cats from various other diagnostic groups,

Figure 1. Distribution of feline serum amyloid A concentrations (mg/L) in 88 cats determined by a human SAA turbidimetric assay in accordance with the presence of an acute-phase response (as indicated by a feline α_1 -acid glycoprotein serum concentration above 0.65 g/L). Horizontal lines indicate median SAA concentrations of the groups

Figure 2. Distribution of feline serum amyloid A concentrations (mg/L) in 88 cats in accordance with the clinically diagnosed group and as determined by a human SAA turbidimetric immunoassay. Horizontal lines indicate median SAA concentration of the individual groups

except those with neoplasia. This is in accordance with previous reports of the overlap performance of feline SAA (Kajikawa *et al.*, 1999; Sasaki *et al.*, 2003). Furthermore, the differences appeared to be of a magnitude that would make the observed imprecision and detection limit acceptable for clinical use.

The cut-off value for AGP used to differentiate between cats with and without an acute-phase response was established independently of the present study (upper 95% confidence limit (Solberg, 1996) of the AGP levels in healthy cats; data including 51 healthy cats in which the AGP serum concentration was measured using the same method as in the present study (Selting *et al.*, 2000)). The previously reported kinetic differences between SAA and AGP (Kajikawa *et al.*, 1999) could cause minor uncertainties regarding the parallel presence of both proteins during an acute-phase response, but the effect was considered to be negligible in the present study. This assumption was sustained by the observed significant correlation of the two acute-phase proteins.

No significant difference was found between the group of infectious/inflammatory disorders and the group of neoplastic diseases, presumably because the group of neoplastic disorders was very heterogeneous in acute-phase response, as also observed in another study (Selting *et al.*, 2000). Thus, the assay demonstrated an overlap performance expected for feline SAA. Variation due to the freezing and storage of samples in the present study should be negligible as SAA has been reported to be stable at -20°C (McDonald *et al.*, 1991) and AGP is reported to be unaffected by freezing (Ganz *et al.*, 1983). Previous reports are available on species-specific determination of feline SAA (DiBartola *et al.*, 1989; Sasaki *et al.*, 2003) and on heterologous determination by means of rabbit anti-canine SAA-antibody-based ELISA (Kajikawa *et al.*, 1996, 1999) and anti-human SAA-antibody-based ELISA (Giordano *et al.*, 2004). However, none of these assays seem to be sufficiently practicable for routine application in terms of availability and automation. To the authors' knowledge there are no previous reports that demonstrate sufficient reliability of an SAA-TIA based on anti-human SAA antibodies for heterologous determination of feline SAA concentration. Analogously to their use in human medicine, feline SAA measurements are suggested to be applicable for diagnostic and monitoring purposes (Kajikawa *et al.*, 1999; Sasaki *et al.*, 2003). The availability of a fast, reliable, readily available and automated assay such as the SAA-TIA should facilitate measurements of feline SAA in diagnostic laboratories and thus routine applicability in feline medicine.

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