Research in Veterinary Science 93 (2012) 649–654

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

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

Acute phase proteins in healthy and sick cats

R.K.C. Kann, J.M. Seddon, J. Henning, J. Meers *

School of Veterinary Science, The University of Queensland, Gatton, QLD 4343, Australia

ARTICLE INFO

Article history:
Received 24 September 2010
Accepted 13 November 2011

Keywords:
Acute phase protein
Serum amyloid A
Haptoglobin
Alpha1-acid glycoprotein
Albumin
Feline

ABSTRACT

Serum acute phase protein concentrations are used as diagnostic, therapeutic and prognostic markers in human and, less frequently, in animal medicine. The aim of this study was to determine how the health status and signalment of the cat are associated with concentrations of acute phase proteins. Generally, medians of the positive acute phase proteins appeared to be higher in sick cats compared to healthy cats. In multivariable regression models, log-transformed serum amyloid A concentration was higher in older cats, in sick and in female cats, while log-transformed α1-acid glycoprotein and haptoglobin concentrations were higher in older cats and were associated with interactions of health status (sick/healthy) and gender (male/female). The data from healthy cats in this study contribute to the limited knowledge of normal reference ranges for this species. This study highlights the potential of acute phase proteins as diagnostic markers in sick cats, but also emphasises that the signalment of the cat needs to be taken into consideration.

1. Introduction

The acute phase response is a complex systemic reaction that occurs in response to both acute and chronic inflammatory conditions such as infection, tissue injury, trauma, surgery, neoplasia or immunological disorders (Baumann and Gauldie, 1994; Gruys et al., 2005). There are three major groups of acute phase proteins based on the magnitude and duration of their response to a stimulus; the major acute phase proteins which are characterised by increases of 10- to 1000-fold in humans or 10- to 100-fold in domestic animals, e.g. C-reactive protein and serum amyloid A; the moderate acute phase proteins which typically demonstrate a 2- to 10-fold increase, e.g. haptoglobin and α-globulins; and the negative acute phase proteins which decrease in concentration following an inflammatory stimulus, e.g. albumin (Steel and Whitehead, 1994; Ceron et al., 2005; Gruys et al., 2005).

Serum acute phase protein concentrations are being increasingly utilised in the assessment of human and animal health to monitor inflammatory processes for diagnostic, prognostic and therapeutic purposes (Buttenschoen et al., 2001; Petersen et al., 2004; Ceron et al., 2005; Dabrowski et al., 2009). The acute phase proteins most commonly analysed with respect to disease association are C-reactive protein, serum amyloid A, α1-acid glycoprotein, haptoglobin and albumin. Measuring acute phase protein levels is of no inherent diagnostic value as changes in these levels indicate only the presence of infectious or inflammatory disease but not the cause of these conditions. However, when interpreted in conjunction with clinical and laboratory parameters, acute phase protein levels can be of assistance in disease diagnosis and management. They can indicate the presence of subclinical inflammation, help to discriminate between acute and chronic disease and help to predict the future course of disease (Horadagoda et al., 1999; Petersen et al., 2004; Ceron et al., 2005). In general, the degree of change is correlated with the severity of disease, being more extreme in more severe and complicated cases. Since the acute phase response develops before stimulation of specific immunological changes it can be utilised as an early marker for disease (Petersen et al., 2004; Ceron et al., 2005).

The use of acute phase proteins has not been widespread in veterinary practice. This is most likely due to the fact that there are insufficient studies concerning the sensitivities and specificities of these tests for the diagnosis of disease, in addition to the fact that there are few commercial veterinary assay kits available and they are relatively expensive. However, there has been some research performed analysing the response of certain acute phase proteins in different diseases: in combination with other diagnostic information such as clinical signs and blood tests, acute phase proteins have been found to be of use in the diagnosis, management and prognosis of diseases such as feline infectious peritonitis, canine inflammatory bowel disease, leishmaniasis, ehrlichiosis, bovine mastitis, equine respiratory disease and canine pyometra (Eckersall and Connor, 1988; Hultén et al., 1999; Eckersall et al., 2001; Martinez-Subiela et al., 2002; Shimada et al., 2002; Jergens et al., 2003; Giordano et al., 2004; Petersen et al., 2004; Dabrowski et al., 2009). Due to the variable response of different acute phase proteins to inflammation and tissue damage, measuring multiple acute phase proteins to monitor the health of an individual is probably more useful than

* Corresponding author. Tel.: +61 7 5460 1839; fax: +61 7 5460 1922.
E-mail address: j.meers@uq.edu.au (J. Meers).

0034-5288/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2011.11.007

assessing a single protein. Ideally a profile of one major, one moderate and one negative acute phase protein should be assessed (Gruys et al., 2005).

The production and response of the various acute phase proteins varies depending on the animal species (Petersen et al., 2004). In general, only one of either C-reactive protein or serum amyloid A is a major acute phase reactant in a given species. In cats, serum amyloid A, α1-acid glycoprotein and haptoglobin are recognised as acute phase reactants whilst C-reactive protein does not seem to be affected by or has only minimal changes in inflammatory conditions (Harvey and Gaskin, 1978; Kajikawa et al., 1999; Sasaki et al., 2003; Ceron et al., 2005). However, there are limited data describing the concentrations of acute phase proteins that are considered 'normal' in healthy cats and those data that are available show variation in the expected normal ranges of individual acute phase proteins (Table 1) (Duthie et al., 1997; Kajikawa et al., 1999; Sasaki et al., 2003; Giordano et al., 2004).

In this study, we evaluated the concentrations of four acute phase proteins in cats of varying health status. There were two objectives to this study. Firstly, we wanted to ascertain whether the health status, gender or age of the cat are associated with the concentration of these acute phase proteins. Secondly, we aimed to generate data on the levels of acute phase proteins that might be expected in normal healthy cats.

2. Materials and methods

2.1. Samples

Samples from 73 domestic cats were used in the study, comprising 48 males, 21 females and four cats for which gender was not recorded. Plasma samples from up to 67 of these cats were tested for the concentration of positive acute phase proteins (serum amyloid A, α1-acid glycoprotein and haptoglobin) and serum samples, available from 58 of the cats, were tested for albumin concentration. The samples were collected from cats that presented to veterinary clinics around Australia ($N = 33$), comprising eight clinics near Brisbane, and one clinic in each of Sydney and Melbourne or from cats surrendered to RSPCA or council animal shelters ($N = 40$) in Brisbane. The research was conducted in accordance with the University of Queensland Animal Ethics Committee (Approval numbers SVS/746/04; SVS/096/06; SVS/320/07). A consent form was signed by owners of all client-owned cats.

For each cat presenting to a veterinary clinic, age, gender and breed were recorded on a project-specific information form, along with clinical history and clinical signs at the time of blood collection. For shelter cats, age was estimated on examination and they were classified as either older or younger than 5 years of age. For these cats, clinical signs present on the day of blood collection were recorded.

All cats in the study were classified as healthy or sick based on the presence or absence of clinical history and clinical signs. Laboratory data (haematology and biochemistry) were available for 15 cats that had been classified as sick on clinical signs or history.

2.2. Sample collection and processing

Blood was collected into standard 1.5 mL EDTA tubes and where possible into standard 1.5 mL plain tubes as well. Occasionally larger EDTA or plain tubes were used. With all tubes, the tube was filled to the minimum required volume. Blood samples were temporarily stored at 4 °C, were packaged according to Australian standards for the transport of biological specimens and transported by courier to our laboratory. For consistency and to allow better comparison of test results, plasma and serum were not removed until 24 h post-collection to allow for travel time for the samples that were received from Sydney or Melbourne. All samples were received within 24 h of collection. Plasma and serum were removed following centrifugation of blood samples at 420*g* for 20 min, then stored at −80 °C until use.

2.3. Determination of feline acute phase protein concentrations

2.3.1. Serum amyloid A

The concentration of serum amyloid A was determined using a solid phase sandwich ELISA kit (Tridelta Development Limited), following the manufacturer's instructions. Plasma samples were assayed in duplicate. Standards of known concentration were used to construct a standard curve. The serum amyloid A concentration of test samples was calculated by interpolation against this standard curve.

2.3.2. Alpha 1-acid glycoprotein

A radial immunodiffusion kit (Tridelta Development Limited) was used to measure α1-acid glycoprotein, following the manufacturer's instructions. Plasma samples were tested in duplicate. A standard curve was made using two standards of known concentrations of α1-acid glycoprotein and the concentration of the samples was determined by comparison of the size of the antigen–antibody precipitin rings with the standard curve.

2.3.3. Haptoglobin

Haptoglobin was measured using a kit (Tridelta Development Limited) following manufacturer's instructions. This kit is based on the detection of a colour change resulting from interactions between haptoglobin, haemoglobin, a chromogen and hydrogen peroxide. Any visibly hemolysed samples, which are generally recognised to contain haemoglobin concentration between 300 and 500 mg/L (Fairbanks, 1982; Stockham and Scott, 2008) were discarded, although the manufacturers state that the kit is accurate with mildly hemolysed plasma samples (containing haemoglobin up to 2.5 g/L) (Tridelta Development Limited). Plasma samples were tested in duplicate and standards of known haptoglobin concentration were used to generate a standard curve which was then used to calculate the haptoglobin concentration in the test samples.

2.3.4. Albumin

Serum albumin concentrations were determined at the University of Queensland Veterinary Clinical Pathology Laboratory using an Olympus analyser which utilises a photometric colour test for the quantitative determination of albumin concentration.

2.4. Statistical analysis

Descriptive analysis included the presentation of means, medians and ranges of acute phase protein concentrations for healthy and sick cats.

Table 1
Ranges or mean ± standard deviation for acute phase proteins in healthy cats reported in the literature.

	Giordano et al. (2004)	Duthie et al. (1997)	Kajikawa et al. (1999)	Sasaki et al. (2003)
Serum amyloid A (μg/mL)	10.21 ± 8.32		16.6 ± 11.4	0.6 ± 1.06
α1-Acid glycoprotein (μg/mL)	1200 ± 620	100–480	244.1 ± 96.1	
Haptoglobin (mg/mL)	1.3 ± 0.64	0.04–3.84	0.416 ± 0.367	