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Establishment of reference interval for SAA in felines.

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Introduction

Acute phase proteins are proteins whose concentration in the blood fluctuates by acute phase reactions when an infection or inflammatory disease has occurred and is initiated locally by the release of cytokines, such as IL-1, IL-6, and TNF-α, from inflammatory cells. In dogs and humans, CRP is widely used as an inflammatory marker. However, no differences have been found in CRP concentrations between clinically normal and diseased felines or those that had undergone surgery [1]. In felines, SAA, haptoglobin and alpha (1)- acid glycoprotein are known as inflammatory marker. SAA increases earlier and more drastically than other two markers [1,2]. We focused on these SAA's features and developedthe FUJI DRI-CHEM IMMUNNO AU CARTRIDGE vf-SAA as a SAA measurement reagent exclusively for felines. Escribano et al. had reported clinical availability of this in-house assay [3]. The purpose of this study is the establishment of the reference interval by using vf-SAA. Furthermore, SAA concentrations were measured in healthy and diseased felines to assess the adequacy of the reference interval.

Material and Methods

All procedures were approved by the Animal Care and Use Committee of Fujifilm Corporation (approval no. A-1-180536 and A-1-190085) before commencing this study.

1) SAA reference interval

The reference interval was established by the following method based on the CLSI (Clinical and Laboratory Standards Institute) guidelines.

1. Criteria of healthy felines

The felines examined at six veterinary hospitals between April 2017 and November 2018 were used for this study. The felines were confirmed to have no clinical symptoms and all biochemical test results are within the reference interval.

test item	ALP	GPT/ALT	TP	BUN	Cre	WBC
unit	U/L	U/L	g/dL	mg/dL	mg/dL	$\times 10^3$/uL
reference intervals	aged >1 year 38 ～ 165 aged <1 year 77 ～ 358	22 ～ 84	5.3 ~ 9.0	17.6 ～ 32.8	0.8 ～ 1.8	5.5 ～ 19.5

2. Sample collection and storage

Plasma samples were collected by venipuncture at each facility by taking 1–1.5 ml of whole blood samples from each feline in a FUJI heparin tube (Fujifilm Corporation, Tokyo, Japan). The tubes were inverted five to six times to mix the contents. The samples were centrifuged immediately after collection at 1,000–1,500g for 10–15 min using commercially available centrifuges. The supernatant (0.5–0.75 mL) was transferred

into a FUJI plain tube (Fujifilm Corporation, Tokyo, Japan) and frozen until use. The heparinized plasma was stored at −80°C until analysis.

3. SAA Measurement

SAA concentrations were measured with a FUJI DRI-CHEM IMMUNO AU10V analyzer (Fujifilm Corporation, Tokyo, Japan) using the FUJI DRI-CHEM IMMUNO AU CARTRIDGE vf-SAA.

4. Statistical procedure

The histogram of the measured values was made, and its distribution was confirmed. When the histogram is normal distribution, the reference interval is set by the parametric method, and when the histogram is non-normal distribution, the reference interval is set by the nonparametric method. The reference interval was calculated by using StssV/Excel2007 version 18 (Chiba Association of Medical Technologists, Chiba, Japan).

2) Measurement of SAA in diseased felines

The felines examined at the University of Tokyo Veterinary Medical Center and the Rakuno Gakuen University Animal Medical Center for medical treatment between March 2017 and November 2018 were considered as diseased felines in this study. SAA concentration was measured in each sample and the results were compared for healthy felines. Sample collection and the measurement of SAA were performed according to the same method described above.

Results

1) SAA reference interval

A total of 83 plasma samples were collected. The median age of the felines was 5.0 years (range, 6 months to 14 years). The 33 male and 50 female felines included mixed breeds (n = 73), Ragdoll (n = 3), American short hair (n = 2), Scottish Fold (n = 2), Somali (n = 1), Norwegian Forest Cat (n = 1), and Main Coon (n = 1). Because the histogram showed a non-normal distribution (Fig. 1), the reference interval was calculated by non-parametric method. The upper limit of this interval was determined to be 5.49 μg/mL. Lower limit was not determined because the calculated result lower than lower limit of the reagent.

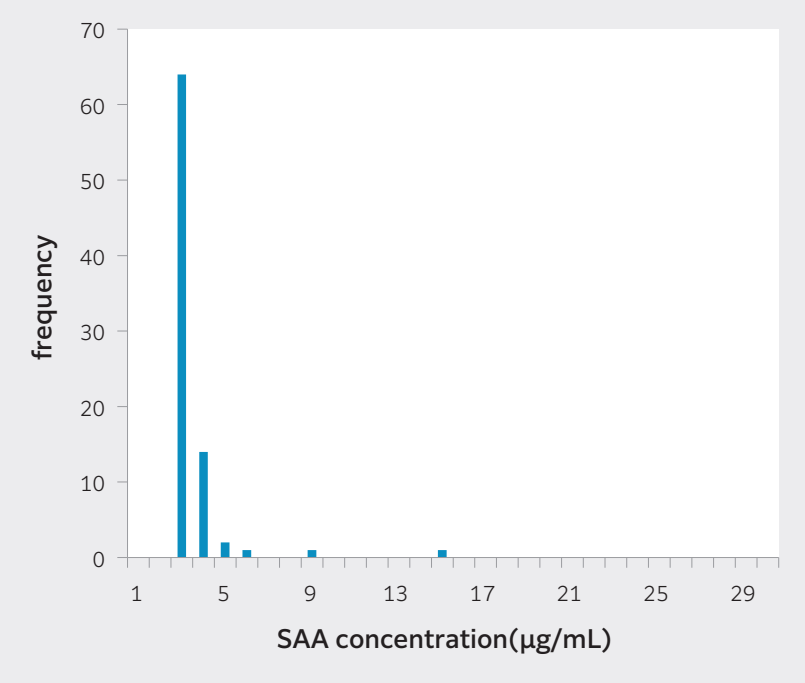

Fig.1　Distribution of SAA concentration of normal felines

2) Measurement of SAA in healthy and diseased felines

A total of 171 diseased felines were analyzed, of which 104 were male and 67 were female. Felines had a median age of 10.0 years (range, 6 months to 20 years). Breeds included mixed (n = 99), Scottish Fold (n = 11), American short hair (n = 9), Japanese bobtail (n = 8), Main Coon (n = 7), Abyssinian (n = 6), Chinchilla (n = 4), Munchkin (n = 4), Norwegian Forrest Feline (n = 3), Russian Blue (n = 3), Bengal (n = 3), Exotic short hair (n = 3), Himalayan (n = 3), British short hair (n = 2), Somali (n = 2), Siamese (n = 1), Ragamuffin (n = 1), Singapore (n = 1), and American curl felines (n = 1). The median SAA concentration obtained from diseased felines were significantly higher than those obtained from normal felines (6.6 μg/mL vs. 2.5 μg/mL) (Fig. 2). Ninety diseased felines (53%) had increased SAA concentrations above the reference interval.

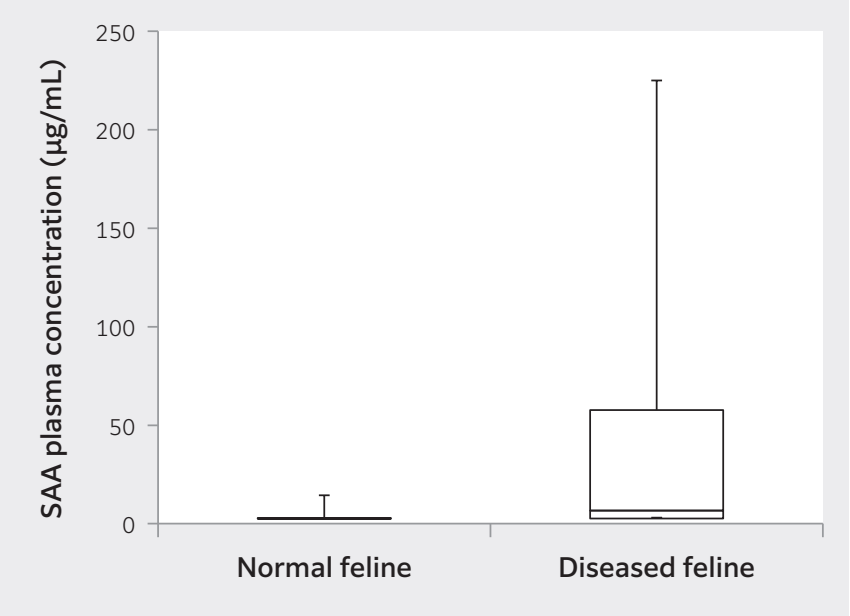

Fig.2 Comparison of SAA concentrations in normal and diseased felines.

Discussion

The upper limit of the reference interval established in this study (5.49 μg/mL) was comparable to that reported by Sasaki et al.[4] and Ishioka et al.[5] SAA concentrations obtained from diseased felines were significantly higher than those obtained from healthy felines. And most of the diseased felines showed increased SAA levels above the reference interval in our study, suggesting that the reference interval was valid in clinically disease monitoring.

References

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