Serum amyloid A (SAA) is an acute-phase protein used as a veterinary clinical test to detect inflammatory disease in cats. In addition to diagnosis, its usefulness in prognostic prediction and as a biomarker for inflammatory diseases is being investigated. The analytical validity of different measurement methods (e.g., FUJI DRI-CHEM IMMUNO AU CARTRIDGE FUJIFILM Tokyo Japan, vf-SAA, and SAA VET test kit Eurolyser Dignostica GmbH Salzburg Austria) has also been confirmed. Currently, measuring SAA concentrations used in many studies is performed using an automated turbidimetric based on a mixture of anti-human SAA-specific monoclonal and polyclonal antibodies assays (LZ-SAA). Recently, the demand for improved specificity and sensitivity of the assay for SAA has necessitated the development of pure monoclonal anti-human antibody assays (VET-SAA). The measurement accuracy of VET-SAA for inflammatory disease was validated using data from a secondary care veterinary hospital population. Nevertheless, the improved utility of switching to VET-SAA in primary care populations has yet to be evaluated. This study compares the utility of VET-SAA with LZ-SAA in clinical practice in primary care hospital populations through SAA measurements in healthy and diseased domestic cats.

OBJECTIVE
Comparing the utility of the anti-human serum amyloid A (SAA)-specific monoclonal and polyclonal antibodies assays (LZ-SAA) with the pure monoclonal anti-human antibody assays (VET-SAA) during clinical practice in primary care hospital populations by measuring SAA measurement in healthy and diseased domestic cats.

ANIMALS
52 healthy and 185 diseased client-owned cats.

METHODS
SAA concentration was measured using different LZ-SAA and VET-SAA measurements for healthy and various diseased cats. Sensitivity, specificity, and accuracy were calculated for each disease.

RESULTS
VET-SAA has higher sensitivity than LZ-SAA for the most common diseases presenting to primary care veterinary hospitals, including chronic kidney disease, tumors, and gingivostomatitis. Our results reveal the capability of detecting low SAA concentrations in healthy and diseased cats using VET-SAA in contrast to LZ-SAA, which found elevations of SAA concentrations only in diseased cats.

CLINICAL RELEVANCE
Our findings indicate that switching to the new VET-SAA instead of the conventional LZ-SAA will likely enhance the diagnostic performance in primary care veterinary hospitals.

Keywords: acute-phase protein, assay, cat, inflammation, serum amyloid A
Methods

Patient population

This study was conducted in accordance with the ethical codes of the Japan Veterinary Medical Association. Written consent was obtained from each cat owner before using the samples collected for this study.

This is a prospective observational study at Yuki Animal Hospital in which 52 client-owned healthy cats and 185 client-owned cats with various medical diseases were recruited from April 2022 through October 2023. Cats that had undergone a health check or had clinical symptoms were randomly selected, whereas cats that had already been diagnosed with a disease or had started treatment were excluded. We performed a physical examination and screening tests on all participating cats, which included a complete blood count, serum biochemistry profile, T4, feline immunodeficiency virus antibody test, and feline leukemia virus antigen test. Additional tests, such as urinalysis, radiography, and ultrasonography, were added as needed for cats suspected of having disease upon notification from their owners.

The healthy group consisted of cats with no clinical signs or abnormalities in the screening tests. The diseased cat group consisted of cats with either clinical signs or abnormalities in any of the tests or both and with or without a definitive diagnosis. Cats with a confirmed diagnosis were sorted or categorized by disease. Twenty-eight cats were randomly sorted from the healthy cats group selected above, age-matched to the diseased cat group, and used for comparison.

Blood collection and quantification

Blood samples for complete blood count and serum or plasma analysis were collected from all cats by venipuncture of the cephalic, saphenous, or jugular veins. All blood samples were placed in tubes with or without an anticoagulant. Serum or plasma was separated from the blood samples within 30 minutes of collection. All samples were analyzed on the day of collection.

Assay for SAA

We obtained SAA concentrations using an automated turbidimetric assay (BioMajesty 6070; JEOL Ltd) based on LZ-SAA and VET-SAA “Eiken” Reagent (Eiken Chemical Co). To control the quality of these reagents, specific calibrators were used for each reagent, and measurements were performed using a dedicated control. The measured values were compared with the displayed ones to confirm their validity. These measurements were performed in a commercial laboratory (Animal Medical Technology). Additionally, the limit of detection and quantification of LZ-SAA and VET-SAA were calculated using cat pool serum of known concentrations as a specimen standard. Clinical and Laboratory Standards Institute testing and calculation methods were utilized as a reference for all specimens.15 The Reference Value Advisor was utilized to perform the reference value calculations.16

Data analysis and statistics

Data were not normally distributed by the Kolomogorov-Smirnov test; therefore, all analyses used nonparametric tests. The comparison of SAA concentrations in cats from the healthy and diseased groups was completed using the Mann-Whitney U test. Results are reported as median (interquartile range [IQR], 25th to 75th percentile) and are represented graphically as Box-Whisker plots. The test’s ability to diagnose the presence or absence of disease was assessed using receiver operating characteristic analysis, which revealed the accuracy of the test. The area under the receiver operating characteristic curve (AUC) and sensitivity and specificity with 95% CIs were calculated. The optimal threshold was determined using the threshold that maximized the sum of sensitivity and specificity. To evaluate the prognostic ability of SAA, we created a survival curve using data from disease groups in which survival or nonsurvival at 30 days was known. Survival curves were generated using the Kaplan-Meier product-limit method and were compared using the log-rank test. To investigate the potential application of LZ-SAA and VET-SAA concentration as a prognostic factor for disease, we performed multivariate analysis (logistic regression analysis), including 12 factors selected by forward selection (age, LZ-SAA or VET-SAA, albumin, blood urea nitrogen, creatinine, glucose, alanine transaminase, hematocrit, white blood cell count, neutrophil count, and feline immunodeficiency virus/feline leukemia virus infections) that are likely to be related to prognosis prediction.2,12,17 Data on these factors were compiled from medical records.

We considered P < .05 significant. All statistical analyses were performed using the EZR Saitama Medical Center (Jichi Medical University), a graphical user interface for R version 4.2.1 (The R Foundation for Statistical Computing). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.18

Results

Assay for SAA

The limit of detection and quantification range of LZ-SAA and VET-SAA measured by automated turbidimetric assay were 0.54 mg/L and 3.85 mg/L and 0.97 mg/L and 3.97 mg/L, respectively. Based on this result, in LZ-SAA, 0.5 mg/L or lower was set as 0 mg/L, and 3.8 mg/L or lower was set as 3.8 mg/L; in VET-SAA, 0.9 mg/L or lower was set as 0 mg/L, and 3.9 mg/L or lower was set as 3.9 mg/L.

Serum amyloid A concentration in healthy cats

The healthy cats group consisted of 25 females (1 sexually intact, 24 neutered) and 27 males (1 sexually intact, 26 neutered). The median (known)
age of the healthy cats was 5.8 years (IQR, 3.5 to 9.8 years), whereas the age of 10 of the healthy cats was unknown. The median age of healthy cats (n = 28) matched for the disease group was 8.3 years (IQR, 5.8 to 10.9 years). The median body weight of the healthy cats group was 4.8 kg (IQR, 3.8 to 5.3 kg). Using the nonparametric method, we determined that the reference values for healthy cats ranged from 0 mg/L (lower limit reference interval [RI]) to 0 mg/L (upper limit RI) for LZ-SAA and from 0 mg/L (lower limit RI) to 4.0 mg/L (upper limit RI) for VET-SAA.

Concentration of SAA in diseased cats

The group of diseased cats (n = 185) consisted of 93 females (24 sexually intact, 69 neutered) and 92 males (13 sexually intact, 79 neutered). The median (known) age of the diseased cats (n = 177) was 10.7 years (IQR, 5.0 to 14.3 years), whereas the age of 8 cats was unknown. The median body weight of the diseased cats was 3.9 kg (IQR, 2.8 to 5.0 kg). We classified the diseased cats into 22 disease categories as follows: chronic kidney disease (n = 28), gastroenteritis (n = 21), tumor (n = 21), upper respiratory tract infection (n = 12), gingivostomatitis (n = 12), lower urinary tract disease (n = 10), pancreatitis (n = 7), feline infectious peritonitis (n = 7), intestinal obstruction (n = 5), traumatic disease (n = 5), diabetes mellitus (n = 5), cardiomyopathy (n = 3), hyperthyroidism (n = 3), hepatitis/cholangitis (n = 2), pneumonia (n = 2), acute kidney disease (n = 2), epilepsy (n = 2), pleural empyema (n = 1), osteoarthritis (n = 1), anal sac abscess (n = 1), *Mycoplasma haemofelis* (n = 1), and other diseases (n = 34).

Figure 1 shows that LZ-SAA and VET-SAA were significantly higher in the diseased group compared to the healthy group (P < .001 and P < .001, respectively). The median values and IQR of LZ-SAA and VET-SAA for the main disease groups are shown in the Table 1.

### Comparison of diagnosability of LZ-SAA and VET-SAA for detecting diseased cats

Test results indicate that LZ-SAA and VET-SAA exhibit different AUC values, at 0.77 (95% CI, 0.73 to 0.80) and 0.83 (95% CI, 0.77 to 0.88), respectively. Figure 2 shows that the sensitivity and specificity of LZ-SAA in diagnosing diseased cats using the highest AUC are measured at 53% and 100% (cutoff, 3.8 mg/L), respectively. The sensitivity and specificity of VET-SAA in correctly identifying diseased and healthy cats using the highest AUC are measured at 67% and 100% (cutoff, 4.0 mg/L), respectively. Therefore, the results reveal a higher AUC for VET-SAA compared to LZ-SAA. The AUC, 95% CI, cutoff value, sensitivity, and specificity for the main disease groups are shown in Table 1.

### Monoclonal and polyclonal-serum amyloid and VET-SAA concentration as a prognostic factor for disease

Diseased cats were followed for at least 30 days. Based on a previous study, survivors and nonsurvivors 30 days after diagnosis were confirmed by medical records or interviews with owners, and all cats were classified into 2 groups: low LZ-SAA (n = 65) or low VET-SAA (n = 76) group and high LZ-SAA (n = 95) or high VET-SAA (n = 84) group with median LZ-SAA (3.8 mg/L) or VET-SAA (8.3 mg/L) concentration cutoff values. Survivors and nonsurvivors were confirmed for 160 cats, of which 128 were survivors and
4

There were 79 survivors in low LZ-SAA group (median 0 mg/L) and 49 in high LZ-SAA group (95.2 mg/L). There were 16 nonsurvivors in both low and high LZ-SAA groups (0 mg/L and 68.8 mg/L, respectively). There were 74 survivors in low VET-SAA group (3.9 mg/L) and 54 in high VET-SAA group (49.9 mg/L). There were 10 nonsurvivors in low VET-SAA group (5.6 mg/L) and 22 in high VET-SAA group (43.8 mg/L). No cats were euthanized among nonsurvivors.

Using univariate analysis, Figure 3 shows that 30-day survival in low LZ-SAA concentration group was not significantly different from that in high LZ-SAA concentration group (P = .22); however, 30-day survival in low VET-SAA concentration group was significantly different from that in high VET-SAA concentration group (P = .007). However, multivariate analysis showed no statistically significant differences in 30-day survival for either LZ-SAA or VET-SAA concentrations (P = .78 and

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Method</th>
<th>Median (mg/L)</th>
<th>IQR (mg/L)</th>
<th>AUC</th>
<th>95% CI</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cutoff (mg/L)</th>
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<tr>
<td>All diseases</td>
<td>185</td>
<td>LZ-SAA</td>
<td>3.8</td>
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<td>9.4</td>
<td>3.9–45.4</td>
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<td>0.77–0.88</td>
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<td>100</td>
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<td>0–3.8</td>
<td>0.64</td>
<td>0.56–0.73</td>
<td>29</td>
<td>100</td>
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<td></td>
<td></td>
<td>VET-SAA</td>
<td>4.9</td>
<td>3.9–14.6</td>
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<td>0.73–0.92</td>
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<td>Gastroenteritis</td>
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<td>0–75.6</td>
<td>0.79</td>
<td>0.68–0.89</td>
<td>57</td>
<td>100</td>
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<td></td>
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<td>0–65.0</td>
<td>0.81</td>
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<td>VET-SAA</td>
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<td>0.69–0.94</td>
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<tr>
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<td>0.78</td>
<td>0.70–0.86</td>
<td>56</td>
<td>100</td>
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<td></td>
<td></td>
<td>VET-SAA</td>
<td>23.1</td>
<td>4.5–57.5</td>
<td>0.87</td>
<td>0.78–0.96</td>
<td>74</td>
<td>100</td>
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</table>

AUC = Area under the receiver operating characteristic curve. LZ-SAA = Anti-human serum amyloid A (SAA)-specific monoclonal and polyclonal antibodies assays. VET-SAA = Pure monoclonal anti-human antibody assays.
Hematocrit was an independent prognostic factor for both LZ-SAA and VET-SAA ($P = .01$ and $P = .01$, respectively).

**Discussion**

In this study, the RIs for healthy cats were set at 0 mg/L for LZ-SAA and from 0 mg/L to 4.0 mg/L for VET-SAA. LZ-SAA for healthy cats all showed 0 mg/L. Sasaki et al.\(^{14}\) reported on SAA concentrations in healthy cats using a direct enzyme-linked immunosorbent assay.\(^{14}\) The SAA concentrations were found to be dispersed between 0 and 4.0 mg/L, with a cutoff value of 3.79 mg/L, indicating that low concentrations of SAA can be detected even in healthy cats. By contrast, the results in our study revealed that SAA in healthy cats is not detectable with LZ-SAA. LZ-SAA concentration in healthy cats measured by LZ-SAA is 0.4 mg/L, 0.6 ± 1.1 mg/L, or 0.14 mg/L,\(^{11,12,19}\) but considering the detection limit, it is actually 0 mg/L. Nevertheless, the limit of detection of LZ-SAA is calculated at 0.54 mg/L by dilution from cat pooled high-concentration serum (similar to the value of 0.38 mg/L reported by Hansen et al.\(^{11}\)). We found LZ-SAA capable of detecting SAA in diseased cats (high-concentration serum or its dilution), whereas VET-SAA can detect SAA in healthy and diseased cats. This may suggest that the SAAs of healthy and diseased cats do not correspond to the same substance. In contrast to LZ-SAA, VET-SAA exhibits a higher sensitivity as it is capable of measuring SAA at low concentrations. The findings suggest that LZ-SAA may increase apparent specificity.

The importance of measuring SAA concentrations in primary care veterinary hospitals is to aid in the early detection of disease at the time of periodic health examination (in asymptomatic cats) and to aid in the diagnosis of disease in cats showing any clinical signs. Although the usefulness of VET-SAA was previously found to be limited to inflammatory diseases,\(^{14}\) it is important for primary care hospitals to assess the efficacy of measuring SAA concentrations for a larger variety of diseases. Herein, we measured and compared VET-SAA and LZ-SAA in cats with various diseases, which were not limited to inflammatory diseases. Our findings demonstrated that VET-SAA has improved sensitivity compared with LZ-SAA for a wide range of diseases, including noninflammatory diseases.\(^{13}\) This result may be useful, for example, in excluding tumors with or without inflammation. We identified a consistently higher AUC of VET-SAA compared to LZ-SAA for many diseases, although the tendency was opposite in the case of a few diseases, which we associated with the higher apparent specificity of LZ-SAA mentioned above. Our findings revealed that a cutoff value of 4.0 mg/L for VET-SAA is appropriate for detecting the presence or absence of diseases in primary care veterinary hospitals, with an extended sensitivity not limited to inflammatory diseases.

We have previously reported that SAA concentrations cannot predict the prognosis of various diseases using primary care facilities as a population.\(^{7}\) In this study, we did not observe long-term prognosis at 6 months or 1 year. However, in this study, it has been shown that neither LZ-SAA nor VET-SAA can predict the prognosis of various diseases after at least 1 month.

Figure 3—Kaplan-Meier curves of anti-human serum amyloid A (SAA)-specific monoclonal and polyclonal antibodies assays (LZ-SAA) and pure monoclonal anti-human antibody assays’ (VET-SAA) concentrations at diagnosis. The univariate analysis shows that LZ-SAA (A) low versus high SAA concentration groups did not differ significantly in survival at 30 days ($P = .23$); however, low VET-SAA (B) differed significantly from that in high SAA concentration group in survival at 30 days ($P = .007$).
Conclusively, our investigation demonstrates the improvement of diagnostic performance in measuring SAA concentrations in primary care veterinary hospitals by switching from the conventional LZ-SAA to the novel VET-SAA. In the future, further clinical usefulness should be verified by measuring VET-SAA for each disease. Further research may help establish the utility of VET-SAA in predicting prognosis for specific disease states.

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Disclosures

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