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Serum alpha 1-acid glycoprotein and interleukin-8 elevations in felines with localized and metastatic tumors

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OBJECTIVE
This study aimed to investigate the expression of acute phase proteins and plasma cytokines in cats with various tumor types and varying metastatic statuses.

ANIMALS
5 clinically healthy cats and 22 cats with neoplastic disease that underwent CT imaging before treatment were enrolled. Patients were grouped based on their tumor types and metastatic status.

METHODS
Blood samples were collected from all cats for general blood analyses before they underwent CT imaging. The remaining plasma sample was frozen for subsequent alpha 1-acid glycoprotein (AGP), serum amyloid A (SAA), and feline cytokine panel measurements. These results were compared with those of healthy cats as well as between metastatic status and tumor types.

RESULTS
Only 4 cats (18%) exhibited elevated SAA levels, whereas 16 (73%) showed elevated AGP levels. AGP was significantly increased in cats with tumors (P = .016), while SAA was not. Only IL-8 showed a significant difference (P = .002) between cats with primary tumors and those with radiologically suspected tumor metastasis.

CLINICAL RELEVANCE
While AGP is a more prominent biomarker than SAA in cats with tumors, a significant elevation of AGP and SAA levels in association with metastasis and specific tumor types could not be identified. Alternatively, further investigation is warranted to evaluate the potential significance of IL-8 in tumor progression and metastasis.

Keywords: alpha 1-acid glycoprotein, cytokine, IL-8, serum amyloid A, tumor

Cancer affects a significant proportion of cats, with a prevalence of approximately 0.5–1% and high mortality rates.[1] The most common types of feline tumors are carcinomas, sarcomas, and lymphomas.[2] Each tumor has its unique etiology, common sites, and oncogenomics. Survival times and treatment methods also vary among different types of tumors.[3] Besides, chronic inflammation can cause changes in the epithelial cytoarchitecture and surrounding stromal components, leading to tumor proliferation, angiogenesis, invasion, and metastasis.[4,5] Chronic inflammation is also believed to be the primary cause of feline injection-site sarcoma, which presents with different histologic variants.[6]

Acute phase proteins (APPs) and cytokines are 2 biomarkers closely associated with inflammation.[5] These proteins are crucial for maintaining homeostasis, promoting tissue repair, and eliminating the

underlying causes of disturbances, such as infections, trauma, and tumors.[7] It is believed that APPs are synthesized in response to proinflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-alpha (TNF-α), primarily in the liver.[8,9] Each species also possesses its own unique major, moderate, and minor APPs. The major positive APPs in cats include serum amyloid A (SAA) and alpha-1-acid glycoprotein (AGP).[10] SAA levels increase within 8 hours after inflammatory stimulation and peak at 24 to 48 hours, often rising 10- to 50-fold.[11] SAA, a cytokine-like protein, is recognized for its role in cell-cell communication and feedback in inflammatory, immunologic, neoplastic, and protective pathways.[12] Increased SAA levels have been observed in several feline diseases, including feline infectious peritonitis, enteritis, renal failure, and neoplasia.[13] The peak of AGP occurs 36 hours after inflammatory stimulation and can increase by up to 2- to 5-fold.[11] While the function of AGP is not fully defined, current knowledge suggests it may be associated with immunomodulation and antiinflammatory effects, such as the inhibition of lymphocyte proliferation.[14] AGP is used to monitor various feline diseases, including feline infectious peritonitis, pancreatitis, feline lower urinary tract disease, and lymphoma.[11] AGP levels are significantly higher in tumor-bearing cats, with no significant differences found among tumor types.[15] In contrast, SAA levels are elevated in non-nasal lymphomas when compared with nasal lymphoma.[16] The potential benefits of the combined use of AGP and SAA in various feline tumors have not been reported.

Cytokines are small proteins secreted by cells that allow for communication and coordination of complex multicellular behaviors. They can be classified into several functional categories, including proinflammatory cytokines (such as IL-1β, IL-6, and TNF-α), chemokines (including CC, CXC, C, and CXXXC chemokines), and antiinflammatory cytokines (such as IL-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13).[17] Proinflammatory cytokines play a key role in activating immune cells and promoting the production and release of additional cytokines.[18] Chemokines, which are small (8–12 kDa) chemotactic cytokines, regulate the promotion and inhibition of angiogenesis and the recruitment of inflammatory cells.[19] Conversely, antiinflammatory cytokines are a group of immunoregulatory molecules that help control the proinflammatory cytokine response.[20] Cytokines play a significant role in both inflammation and cancer[17] and have a close relationship with cancer development and metastasis.[21]

With a suspected association between tumors and inflammation, this study aimed to investigate the expression of APPs and plasma cytokines in cats with various tumor types and varying metastatic statuses, to explore the feasibility of clinical applications and provide further information on tumorigenesis.

Methods

Animals

In this prospective clinical trial, 5 client-owned clinically healthy domestic shorthair cats and 22 client-owned cats (18 domestic shorthair, 2 American shorthair, 1 Chinchilla, and 1 Scottish fold) with neoplastic diseases were recruited from a referral medical imaging center, UniCore Animal Hospital, Taipei City, Taiwan. The signalments of all cats recruited for this study were collated **(Table 1)**. The inclusion criteria for clinically healthy cats were the absence of known ongoing diseases, discomfort, abnormal findings on physical examination, availability of blood test results (including complete blood count and common serum biochemistry profile), and chest and abdominal radiography. The inclusion criteria for cats with neoplastic diseases were as follows: cats diagnosed with malignant tumors through histopathological or cytological examination before a

Table 1—Demographic data, numbers, metastatic status, and tumor types in cats.

	Clinically healthy	**Tumor**		
Characteristic	**(n = 5)**	**(n = 22)**		
Age (years)	9 (8–14)	10 (3–17)		
Sex				
Male (n)	2	13		
Female (n)	3	9		
Weight (kg)	5.2 (2.8–9.1)	4.6 (2.7–6.4)		
		Primary tumor (n = 15)	**Radiologically Suspected metastasis (n = 7)**	
Age (years)		10 (3–17)	10 (6–17)	
Sex				
Male (n)		5	8	
Female (n)		2	7	
Weight (kg)		4.8 (3.7–6.1)	4.6 (2.7–6.4)	
		Carcinoma (n = 12)	**Sarcoma (n = 6)**	**Lymphoma (n = 4)**
Age (years)		13.5 (6–17)	10 (3–14)	8.5 (7–15)
Sex				
Male (n)		7	3	3
Female (n)		5	3	1
Weight (kg)		4.8 (2.9–6.1)	4.5 (3.3–6.4)	4.6 (2.7–5.5)

Data are presented as medians (ranges) or absolute numbers.

specific antineoplastic treatment and those who had undergone CT scanning to ascertain the development of metastasis. Cats with neoplasia and comorbid severe liver dysfunction or hypoalbuminemia were excluded. Malignant tumors were classified as carcinomas, sarcomas, or lymphomas. The presence of suspected metastasis was assessed through a consensus reached by a radiologist (L.S.L) with 15 years of experience and 2 third-year radiology residents. The evaluation was based on careful analysis of the CT images obtained, looking for evidence of lymph node enlargement or abnormal enhancement and tumors in multiple organs such as the lungs, liver, spleen, muscles, and other relevant areas.

This study was approved by the Institutional Animal Care and Use Committee of the National Pingtung University of Science and Technology, Taiwan (NPUST-110-076). Study-specific informed consent was obtained at the hospital.

Blood collection

Blood samples were collected from all cats by cephalic venin or internal saphenous venipuncture with 23-gauge needles. Whole blood (2 mL) was collected and loaded into ethylenediaminetetraacetic acid and heparin anticoagulant tubes for complete blood counts and serum biochemistry profiles, respectively. The remaining plasma samples from the heparin anticoagulated blood were stored at −80 °C to measure AGP, SAA, and cytokine levels within 6 months.

SAA and AGP measurements

SAA levels were measured using a feline-specific commercial kit (fSAA 2.0; VCheck, Korea). Exactly 5 µL of plasma was required for each test. The linear measurement range of the assay was 5 to 200 mg/L. Based on the manufacturer's instructions, values below 5 mg/L were considered negative results.

AGP levels were measured using a commercial feline-specific kit (fAGP; Point Reader™ V, Japan). Exactly 10 µL of plasma was used for each test. The linear measurement range of the assay was 400 to 3,000 mg/L. Although the reference interval for healthy cats was not provided by the manufacturer, according to the reference interval set by our laboratory, results below 780 mg/L are considered within the normal range, and the reference is comparable to that of a previous study.[22]

All assays for AGP and SAA were performed by the same operator, ensuring consistency and minimizing potential variability in the results due to operator differences.

Measurement of plasma cytokine levels

The concentrations of plasma cytokines and chemokines were measured using a feline-specific, commercial, antibody-coated, microsphere-based multiplex cytokine immunoassay (Fctyomag-20K Milliplex Map Feline Cytokine/Chemokine Magnetic Bead Panel, Premix 19 Plex kit; Merck Millipore Corporation) that can quantify 19 cytokines (sFas, Flt-3 ligand, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12-p40, IL-13, IL-18, KC, MCP, PDGF-BB, RANTES, SCF, SDF-1, and TNF-α) simultaneously. Exactly 25 µL of each patient's plasma was used for each test, and the procedures followed the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using commercial statistical software (GraphPad Prism 8.0; GraphPad Software, Inc). For statistical analysis, samples with AGP and SAA results outside the linear range of the assay (<400 or >3,000 mg/L; <5 or >200 mg/L) were assigned values of 400 or 3,000 mg/L and 5 or 200 mg/L, respectively. Descriptive analyses were reported as medians and ranges. AGP, SAA, and plasma cytokine levels were compared using the nonparametric Mann–Whitney U test between (1) clinically healthy cats and cats with tumors and (2) cats with primary tumors and those with radiologically suspected tumor metastasis. Differences between tumor types were compared using the nonparametric Kruskal–Wallis test, followed by Dunn's post hoc test. Statistical significance was set at $P < .05$. Bonferroni corrections were applied to control the overall Type I error for multiple comparisons of plasma cytokines and the threshold for significance was adjusted to $P < .0026$ (0.05/19).

Results

Signalments of cats with different tumor types

Fifteen cats (68%) were diagnosed with primary tumors and showed no radiologically suspected metastasis. They had a median age of 10 years (range = 3–17 years). Seven cats (32%) were radiologically suspected of having metastasis; their median age was 10 years (range = 6–17 years). Twelve (55%) cats had carcinomas, with a median age of 13.5 years (range = 6–17 years); 6 (27%) had sarcomas, with a median age of 10 years (range = 3–14 years); and 4 (18%) had lymphomas, with a median age of 8.5 years old (range = 7–15 years) (Table 1). The median values of complete blood count and biochemistry were within the reference ranges.

SAA and AGP expressions in feline patients

Abnormal SAA and AGP levels in cats with tumors were observed **(Table 2)**. Of the cats studied, 4 (18%) exhibited elevated SAA levels, while 16 (73%) showed elevated AGP levels. Cats with high SAA levels also

Table 2—Abnormal serum amyloid A and alpha 1-acid glycoprotein levels in cats with tumors.

Protein	AGP(+)	AGP(−)	Total
SAA(+)	4 (18%)	0 (0%)	4 (18%)
SAA(−)	12 (55%)	6 (27%)	18 (82%)
Total	16 (73%)	6 (27%)	22 (100%)

Values above reference limits are represented as "+", while values below the reference limits are represented as "−."

AGP = Alpha 1-acid glycoprotein. SAA = Serum amyloid A.

had high AGP levels. Additionally, 12 cats (55%) had normal SAA but elevated AGP levels. Six cats (27%) did not show elevated levels of SAA or AGP.

AGP levels

AGP concentrations were within the reference value in all clinically healthy cats. Median AGP concentrations in clinically healthy cats and cats with tumors were 519.9 mg/L (range = 400–721 mg/L) and 1621.6 mg/L (range = 400–3,000 mg/L), respectively. In cats with primary tumors and radiologically suspected metastasis, median AGP concentrations were 1568.6 mg/L (range = 400–3,000 mg/L) and 1779.4 mg/L (range = 442.5–3,000 mg/L), respectively; in cats with carcinoma, sarcoma, and lymphoma, the concentrations were 1734.2 mg/L (range = 400–3,000 mg/L), 1299.4 mg/L (range = 496.8–1779.4 mg/L), and 2592.4 mg/L (range = 442.5–3,000 mg/L), respectively. A significant difference was only noted between clinically healthy cats and cats with tumors (P = .016), but not between metastatic status (P = .639) or tumor type (P = .476).

SAA levels

SAA concentrations were below the linear range of the assay in all clinically healthy cats and

Table 3—The plasma cytokine panels of clinically healthy cats and cats with tumors.

	Concentration (pg/mL)				
Cytokine	**Clinically healthy**		**Tumor**		***P* value**
Fas	3.0	(2.2–4.7)	3.5	(2.7–111.4)	.136
Flt-3L	58.6	(21.3–75.9)	58.9	(17.1–124.8)	.891
GM-CSF	4.4	(3.3–5.5)	5.7	(3.3–32.3)	.320
IFNr	58.9	(26.7–171.4)	132.0	(36.3–2702.0)	.133
IL-1b	8.3	(6.4–15.0)	11.4	(6.8–128.6)	.191
IL-2	7.4	(6.9–31.7)	8.6	(6.9–101.3)	.186
PDGF-BB	85.2	(58.4–170.6)	71.5	(52.2–369.0)	.916
IL-12p40	134.5	(87.6–267.3)	154.3	(43.3–844.3)	.639
IL-13	8.8	(8.2–33.0)	24.8	(7.5–92.2)	.182
IL-4	33.6	(20.5–244.3)	266.5	(31.1–3208.0)	.019
IL-6	34.2	(23.9–106.7)	56.9	(28.1–1314.0)	.161
IL-8	6.7	(4.1–11.0)	16.4	(5.1–479.1)	.038
KC	1.3	(0.5–1.4)	2.3	(0.5–62.4)	.264
SDF-1	628.0	(321.5–998.2)	1155.0	(16.0–2237.0)	.032
RANTES	12.9	(5.9–30.3)	11.7	(3.5–63.5)	.939
SCF	64.2	(50.4–102.8)	98.3	(50.4–408.9)	.067
MCP-1	472.1	(60.9–1466.0)	1401.0	(149.3–5837.0)	.132
TNF-α	0.2	(0.0–4.8)	0.3	(0.0–471.0)	.457
IL-18	50.4	(47.5–178.3)	106.8	(47.5–1996.0)	.110

Data are presented as medians (ranges).

Table 4—The plasma cytokine panels of the cats with primary tumors and radiologically suspected metastatic tumors.

	Concentration (pg/mL)				
Cytokine	**Primary tumor**		**Radiologically metastatic tumor**		***P* value**
Fas	3.5	(3.1–4.3)	3.4	(2.7–111.4)	.664
Flt-3L	68.4	(17.1–105.1)	56.7	(19.9–124.8)	.958
GM-CSF	5.8	(4.6–9.0)	5.7	(3.3–32.3)	.290
IFNr	95.6	(52.8–368.8)	164.9	(36.3–2702.0)	.717
IL-1b	8.2	(7.3–24.2)	12.5	(6.8–128.6)	.417
IL-2	8.4	(6.9–15.8)	8.9	(6.9–101.3)	.797
PDGF-BB	81.7	(61.6–306.4)	69.8	(52.2–369.0)	.189
IL-12p40	174.0	(62.1–339.9)	134.5	(43.3–844.3)	.905
IL-13	31.1	(9.2–56.1)	21.0	(7.5–92.2)	.490
IL-4	252.8	(36.1–1069.0)	280.3	(31.1–3208.0)	.694
IL-6	37.4	(28.1–236.1)	81.1	(28.9–1314.0)	.230
IL-8*	7.6	(5.1–13.6)	21.0	(6.1–479.1)	.002
KC	0.7	(0.5–62.4)	3.7	(0.5–21.9)	.340
SDF-1	1185.0	(16.0–1884.0)	1033.0	(519.9–2237.0)	.437
RANTES	10.3	(4.6–12.4)	13.1	(3.5–63.5)	.045
SCF	93.9	(54.7–132.5)	102.8	(50.4–408.9)	.642
MCP-1	970.8	(541.6–2673.0)	1962.0	(149.3–5837.0)	>.999
TNF-α	0.1	(0.1–7.6)	1.5	(0.0–471.0)	.341
IL-18	135.2	(50.4–875.8)	82.0	(47.5–1996.0)	.378

Data are presented as medians (ranges).
*P < .0026.

Table 5—The plasma cytokine panels of the cats with different tumor types.

	Concentration (pg/mL)						
Cytokine	**Carcinoma**		**Sarcoma**		**Lymphoma**		***P* value**
Fas	3.2	(2.9–13.3)	4.3	(3.5–111.4)	3.1	(2.7–3.9)	.041
Flt-3L	63.1	(20.8–106.9)	43.0	(17.1–96.5)	90.5	(43.1–124.8)	.159
GM-CSF	5.4	(3.6–17.0)	6.8	(4.6–32.3)	4.0	(3.3–7.6)	.086
IFNr	97.3	(36.3–2702.0)	254.2	(55.8–502.4)	116.7	(55.8–202.2)	.717
IL-1b	10.7	(7.0–128.6)	19.3	(7.9–30.1)	9.2	(6.8–13.6)	.231
IL-2	8.6	(6.9–100.5)	10.4	(8.4–101.3)	7.7	(6.9–9.4)	.108
PDGF-BB	69.8	(61.6–369.0)	96.2	(69.8–306.4)	102.1	(51.2–223.1)	.224
IL-12p40	154.3	(43.3–844.3)	97.1	(59.0–206.6)	496.3	(203.1–723.4)	.027
IL-13	20.8	(8.2–92.2)	31.7	(14.3–56.1)	9.1	(7.5–41.3)	.184
IL-4	63.2	(31.1–3208.0)	378.1	(252.8–566.5)	314.8	(95.6–1069.0)	.255
IL-6	40.7	(31.1–1314.0)	153.0	(28.1–261.7)	56.9	(28.9–110.9)	.750
IL-8	18.8	(5.5–479.1)	15.8	(5.1–154.3)	9.1	(6.6–28.1)	.657
KC	3.3	(0.46–17.8)	1.1	(0.6–21.9)	2.5	(0.6–62.4)	.990
SDF-1	1070.0	(16.0–1573.0)	1314.0	(638.8–2237.0)	955.3	(520.0–1185.0)	.234
RANTES	11.1	(3.5–55.3)	11.5	(8.7–17.5)	32.1	(11.2–63.5)	.156
SCF	107.8	(54.7–408.9)	92.3	(72.9–135.2)	88.7	(50.4–270.8)	.841
MCP-1	796.1	(149.3–5837.0)	2229.0	(541.6–3146.0)	990.7	(149.3–2175.0)	.420
TNF-α	0.2	(0.0–471.0)	1.2	(0.0–10.3)	0.2	(0.0–5.5)	.935
IL-18	107.6	(47.5–1996.0)	217.4	(82.0–875.8)	59.4	(56.3–135.2)	.137

Data are presented as medians (ranges).

were recorded as 5.0 mg/L. The median concentration of SAA in cats with tumors was 5.0 mg/L (range = 5.0–51.7 mg/L). In cats with primary tumors and radiologically suspected metastasis, median SAA concentrations were 5.0 mg/L (range = 5.0–8.3 mg/mL) and 5.0 mg/L (range = 5.0–51.7 mg/mL), respectively. In cats with carcinomas, sarcomas, and lymphoma, the median concentrations were 5.0 mg/L (range = 5.0–51.7 mg/L), 5.0 mg/L (range = 5.0–5.0 mg/L), and 5.0 mg/L (range = 5.0–8.3 mg/L), respectively. Elevated SAA concentration was noted only in 4 cats with concurrent high AGP levels, including 1 cat with primary lymphoma (SAA, 8.3 mg/L; AGP, 3,000 mg/L) and 3 cats with radiologically metastasis-suspected carcinoma (SAA, 16.8, 24.3, and 51.7 mg/L; AGP, 1059.2, 3,000, and 3,000 mg/L, respectively). No significant differences in SAA were noted between clinically healthy cats and cats with tumors (P = .561), metastasis status (P = .673), or tumor type (P = .412).

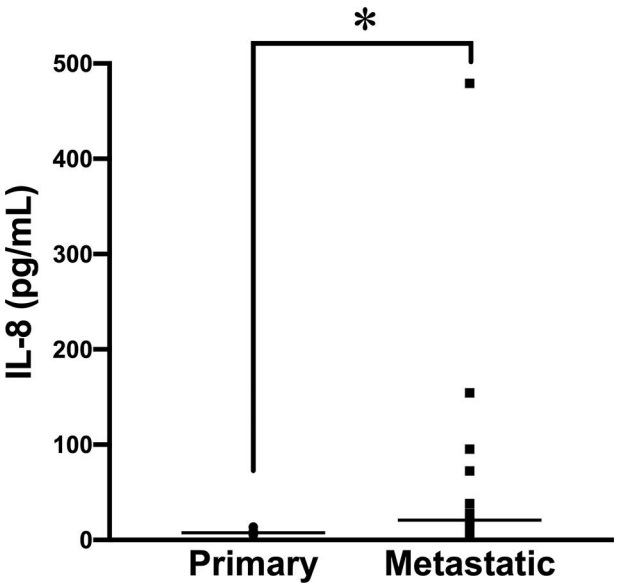

Figure 1—Serum Interleukin (IL)-8 concentration in cats with neoplasia, comparing those with metastatic disease to those without metastases. The concentration of Interleukin-8 was found to be higher in cats with radiologically suspected metastatic tumors compared with cats with primary tumors. *P < .0026.

Cytokines

Plasma cytokine levels in both groups are summarized **(Tables 3, 4, and 5)**. Among all 19 cytokines, only IL-8 levels were significantly higher (P = .002) in cats with tumors and radiologically suspected metastasis than in cats with primary tumors **(Figure 1)** after applying the Bonferroni correction.

Discussion

This study evaluated the levels of AGP, SAA, and various plasma cytokines in clinically healthy cats and cats with tumors. To our knowledge, this study is the first to investigate APPs and plasma cytokines together in cats with tumors. The results demonstrated a significant increase in AGP levels in cats with tumors and significantly higher IL-8 levels in cats with radiologically suspected metastatic tumors than in cats with primary tumors.

In this study, the AGP concentration was significantly higher in cats with tumors than in healthy cats. Therefore, inflammatory stimuli might have increased in these cats. Nonetheless, only a few cats with tumors had elevated SAA levels, without any statistical differences observed compared with healthy cats. This discrepancy can be attributed to several factors. This could be due to the use of equipment with an SAA detection limit of 5 mg/L. Previous studies have reported that the reference

levels of healthy cats are below 1.03 mg/L,[13,23,24] indicating that the lower sensitivity of our equipment might mask significant differences within undetectable levels. Although the high sensitivity of SAA could detect minor inflammatory stimuli at low levels, the mean SAA levels of cats with tumors could still be higher than 4 mg/L, even when using equipment with high sensitivity.[25] Further studies are needed to comprehensively explore the potential clinical benefits of low SAA levels in cats with tumors. Second, the increase in SAA levels occurred faster than the increase in AGP levels in cats experiencing acute inflammatory stimulation. A previous study indicated that feline SAA and AGP levels were notably elevated within 24 hours.[25] However, SAA returned to normal levels, whereas AGP remained high, even after 96 hours. Therefore, the timing of blood collection may be crucial for detecting abnormal SAA levels in certain cases. Furthermore, previous studies indicated that AGP exhibited a higher diagnostic value than SAA for feline infectious peritonitis, a disease characterized by chronic inflammation,[26] and AGP levels were also increased in chronic inflammatory diseases such as chronic kidney disease and chronic gingivostomatitis.[27,28] Thus, elevated AGP levels may indicate chronic inflammation resulting from a tumor, which might not be easily discernible through changes in SAA levels. Third, in addition to its response to inflammatory stimulation, endogenous AGP could be elevated through an antiinflammatory effect by inhibiting inflammation mediated by macrophages.[29] AGP may play a role in tumorigenesis by interacting with tumor-associated macrophages.[30] Consequently, AGP levels could be higher in patients with tumors.

Four cats exhibited increased levels of both AGP and SAA. Two cats had primary tumors, whereas the remaining cats had metastatic tumors. The first case involved bladder transitional cell carcinoma complicated by peritonitis, a dilated unilateral renal pelvis and ureter, and mesenteric lymph node metastasis. In the second case, the cat presented with a rounded pelvic primary lymphoma with an ill-defined margin, which caused compression of the descending colon, resulting in constipation. The third case had primary renal carcinoma complicated by peritonitis. In the fourth case, facial squamous cell carcinoma with lymphatic metastasis occurred, which caused regional bone lysis in the mandibular, pterygoid, zygomatic, and temporal bones. Although notable inflammation could be attributed to peritonitis and invasive bone lysis in 2 of the cats, the precise origin of the severe inflammation in the remaining cases remains unidentified.

In contrast, 6 cats had normal AGP and SAA levels. Three cats had primary tumors, whereas the remaining cats had metastatic tumors. The primary tumors were nasal carcinoma in 1 cat and feline injection-site fibrosarcoma in 2 cats. The cats with metastatic tumors had pancreatic adenocarcinoma, multicentric lymphoma, and mammary gland adenocarcinoma. Considering these findings, the noninclusive results between APP levels and metastatic information and tumor types indicate that changes in AGP and SAA could be poor indicators of these parameters. Although a previous investigation demonstrated that AGP and SAA levels could serve as useful markers for monitoring antineoplastic treatment in cats with lymphoma,[23] we could not assess these benefits in our study because of its design, which involved collecting blood samples only at the beginning of treatment.

Lymphomas, sarcomas, and carcinomas are different types of tumors arising from distinct cell sources: lymphoreticular cells, connective tissues, and epithelial cells, respectively. These tumors have varying incidence, survival, and metastatic rates. Although lymphoma is the most common tumor type in cats, only 4 cases were recruited in this study. The low representation might be due to the recruitment criterion that only feline patients who had undergone CT examinations were enrolled. As lymphoma responds better to chemotherapy than other tumor types, these patients were likely treated with chemotherapy alone. Currently, information regarding APPs in cats with various types of tumors is limited.[15,16] Consistent with previous findings regarding AGP,[15] no significant differences were observed between the different types of tumors in our study. This lack of significant variation may be attributed to the relatively low specificity of APPs and plasma cytokines, as they tend to increase in biologically aggressive diseases rather than specific tumor types.[16]

Cancer development and progression have been correlated with cytokines, and show prognostic potential in humans.[31] The circulating levels of various cytokines also undergo changes across different types of tumors in humans. A previous report has also indicated a significant increase in SDF-1 levels in cats with mammary carcinoma metastasis.[32] Nevertheless, research on cytokine profiles in cats with various tumors remains limited. Therefore, to our knowledge, this is the first comprehensive study to report plasma cytokine expression in cats with tumors.

Before Bonferroni correction, elevated levels of IL-4, IL-8, and SDF-1 were observed in individuals with tumors, with higher levels of IL-8 and RANTES in those radiologically suspected of having tumor metastasis. Differences in Fas and IL-12p40 levels among tumor types were also noted. However, following the Bonferroni correction, only IL-8 showed a significant difference between cats with primary tumors and those with radiologically suspected tumor metastasis. Several potential explanations can be proposed to account for the lack of significance in most cases. First, while the Bonferroni correction is commonly applied to mitigate the occurrence of Type I errors in multiple statistical tests, it comes with the risk of increasing Type II errors.[33] It is possible that differences in the expression of these plasma cytokines exist but require a larger sample size to be demonstrated. Second, the limited number of participants in this study and individual variability could have compromised the statistical power of the tests. For example, the range of SDF-1 levels in healthy cats overlapped with that of cats

with tumors, resulting in the loss of significance after correction.

IL-8, also known as CXCL8, is a proinflammatory CXC chemokine that is crucial for regulating inflammation within the tumor microenvironment. IL-8 signaling stimulates angiogenic responses in endothelial cells, enhances the survival and proliferation of both cancer and endothelial cells, and facilitates the migration of cancer cells, infiltrating neutrophils and endothelial cells to the tumor site.[34] In dogs with mammary gland tumors, a significant increase in the circulating levels of IL-8 has been observed. Furthermore, higher tumor grading is associated with elevated IL-8 concentrations, establishing it as a diagnostic and prognostic marker for canine mammary gland tumors.[35] IL-8 may be produced by feline oral squamous cell carcinoma[36]; however, the role of IL-8 in metastasis in cats has not been reported. Our study yielded comparable results, with cats with radiologically suspected metastasis displaying a significant increase in IL-8 concentrations.

This study had some limitations. It utilized a small sample size, which restricted the conclusions drawn. Additionally, owing to the small number of cases, the population of cats with different types of cancer was even smaller, and we did not analyze the various types of cancer in this study. Second, the metastatic status was determined based on radiographic information rather than pathological examination. Therefore, there is a potential bias in selecting the "true" metastatic patients, which may have interfered with the statistical results. Third, APPs are primarily produced by the liver, and as such, cats with severe liver impairment were excluded from this study. The applicability of our study's results to cats with liver dysfunction remains unknown. Finally, the data were collected at a referral medical imaging center that did not provide further treatment. This resulted in a lack of data on APPs and plasma cytokine levels after treatment.

In summary, we found that cats with cancer had significantly higher serum AGP levels than clinically healthy cats, indicating that AGP may serve as a more prominent diagnostic biomarker for cancer than SAA. Nevertheless, we did not observe significant alterations in the levels of either APP concerning the severity and classification of tumors. Our findings emphasize the significance of IL-8 in tumor progression and metastasis, and further investigation is warranted. Although cytokine tests may take longer to produce results in clinical practice, they may provide useful cancer information. Future research should consider the potential roles of APPs and plasma cytokines when interpreting biomarkers for diagnosing and monitoring cancer progression in cats. By analyzing APPs and plasma cytokines, we may better understand the inflammatory processes involved in feline cancer and potentially improve diagnostic and treatment strategies.

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