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Evaluation of serum pentraxin–3 (PTX–3) levels in cats with neoplasia

Evaluación de los niveles séricos de pentraxina–3 (PTX–3) en gatos con neoplasias

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ABSTRACT

Pentraxin–3 is a multifunctional protein implicated in human oncology due to its roles in regulating inflammation, angiogenesis, and tumor progression. However, its potential as a biomarker in feline oncology remains insufficiently investigated. This study evaluated serum Pentraxin–3 levels in cats with feline injection-site sarcoma and their association with histopathological features. A cohort of 14 cats was included, comprising seven clinically healthy controls and seven with subcutaneous sarcomas. Histopathological examination identified fibrosarcoma (n = 3) and pleomorphic sarcoma (n = 4). Cats with fibrosarcoma were generally characterized by smaller, well-demarcated lesions (< 6 cm), with serum Pentraxin–3 levels ranging from 31 to 89 ng·mL^{-1}. In contrast, cases of pleomorphic sarcoma exhibited infiltrative, poorly defined tumors and significantly higher Pentraxin–3 levels (90–530 ng·mL^{-1}). These findings suggest that serum Pentraxin–3 may reflect tumor burden, histopathological subtype, and biological aggressiveness in feline injection-site sarcoma. However, due to the limited sample size, larger-scale studies are necessary to confirm this association.

Key words: Feline injection-site sarcoma; Pentraxin–3; biomarker; fibrosarcoma; pleomorphic sarcoma

RESUMEN

La pentraxina–3 es una proteína multifuncional implicada en la oncología humana debido a su papel en la regulación de la inflamación, la angiogénesis y la progresión tumoral. Sin embargo, su potencial como biomarcador en la oncología felina aún no ha sido suficientemente investigado. En este estudio se evaluaron los niveles séricos de La pentraxina–3 en gatos con sarcoma felino del sitio de inyección y su asociación con las características histopatológicas. Se incluyó una cohorte de 14 gatos, compuesta por siete controles clínicamente sanos y siete con sarcomas subcutáneos. El examen histopatológico identificó fibrosarcoma (n = 3) y sarcoma pleomórfico (n = 4). Los gatos con fibrosarcoma se caracterizaron generalmente por lesiones más pequeñas y bien delimitadas (< 6 cm), con niveles séricos de La pentraxina–3 entre 31 y 89 ng·mL^{-1}. En contraste, los casos de sarcoma pleomórfico presentaron tumores infiltrativos y mal delimitados, junto con niveles de La pentraxina–3 significativamente más elevados (90–530 ng·mL^{-1}). Estos hallazgos sugieren que la La pentraxina–3 sérica podría reflejar la carga tumoral, el subtipo histopatológico y la agresividad biológica en el sarcoma felino del sitio de inyección. No obstante, debido al tamaño limitado de la muestra, se requieren estudios a mayor escala para confirmar esta asociación.

Palabras clave: Sarcoma felino en el sitio de inyección; Pentraxina–3; biomarcador; fibrosarcoma; sarcoma pleomórfico

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INTRODUCTION

The use of inflammatory markers, particularly in oncology, has gained increasing importance for diagnosis, prognosis, and therapeutic management. Pentraxin–3 (PTX3), classified within the long pentraxin subgroup of acute-phase proteins, functions as a crucial mediator within the innate immune network and is expressed by various cell populations, including myeloid, endothelial, and stromal cells [1, 2]. Unlike short pentraxins (e.g., C-Reactive Proteins), PTX–3 serves as a more specific indicator of local inflammatory activity due to its cellular sources and rapid induction at sites of inflammation [3, 4]. Notably, PTX–3 is not synthesized by T or B lymphocytes, highlighting its distinct regulation in immune responses [1, 5]. Research into the role of pentraxins in neoplastic development is ongoing in both human and veterinary medicine and requires further clarification [5].

Feline injection-site sarcomas (FISS) are aggressive mesenchymal tumors characterized by a high recurrence rate. Fibrosarcoma represents the most common histological subtype; however, other variants, including pleomorphic sarcoma, myofibroblastic sarcoma, and chondrosarcoma, have also been documented [6, 7, 8]. FISS typically occurs at injection sites, most notably in the interscapular region. The latency period between injection and tumor development varies widely in the literature, ranging from several months to several years [9, 10, 11]. Furthermore, factors such as the type and frequency of injections have been reported to play a role in the pathogenesis of FISS [11].

In the pathophysiology of inflammation-associated neoplasm, chronic inflammation serves as a critical component of the tumor microenvironment, actively promoting neoplastic progression and growth [12]. PTX3 is thought to contribute to tumor-associated inflammation by modulating inflammatory signaling pathways and influencing leukocyte dynamics through complement system interactions [12, 13].

Unlike conventional acute-phase proteins, PTX–3 demonstrates a tissue-specific response to local damage, making it particularly valuable for monitoring tumor-related inflammatory processes [12, 13, 14]. Despite these established associations, no studies to date have investigated serum PTX–3 alterations in FISS, highlighting a significant gap in the current understanding of this biomarker's role in Veterinary oncology.

This study therefore aimed to describe the histopathological characteristics of post-vaccinal subcutaneous tumors and to evaluate its association with serum PTX–3 in affected cats (*Felis catus*).

MATERIALS AND METHODS

The study cohort comprised client-owned cats of various breeds, genders, and ages admitted to the Teaching Hospital of the Faculty of Veterinary Medicine, Aksaray University, Türkiye. Subjects were allocated into two groups: Group C included 7 clinically healthy cats presented for routine health examinations and Group T had 7 cats with post-vaccinal subcutaneous masses of varying size. Prior to enrollment, written informed consent was obtained from all pet owners.

Radiographic and ultrasonographic examination

Preoperative assessment for Group T comprised standardized ultrasonography (B-mode and color Doppler; Mindray Z6 Vet, China) and two-view thoracic radiography (right lateral and ventrodorsal; Hasvet 838R X-ray machine, China and Fujifilm CRI 392 reader, Japan). Radiographs were systematically evaluated to determine the primary mass's location and extent, identify local tissue invasion, and screen for intrathoracic metastasis.

Laboratory examination

Alla cats were blood sampled by aseptically vein puncture from the cephalic into plain tubes for biochemistry analyses (Mnchip pointcare v3, China) and EDTA treated tubes for complete blood count (Mindray BC2800 Vet, Auto Hematology Analyzer, China). Blood samples were centrifuged (Hasvet LC–04B, China) at 2325 × g for 10 min and serum was harvested. Following biochemical measurements, the remaining serum was stored at –80°C (Nuve DF590, Türkiye) until PTX–3 analysis.

Serum PTX–3 concentrations were measured using feline specific commercial ELISA kits (ELK Biotechnology®, China) as instructed by the manufacturer.

Surgical treatment

Before surgery, each cat underwent general anesthesia using medetomidine HCl (80 $\mu g \cdot kg^{-1}$, IM; Domitor®, Finland) and ketamine HCl (5 $mg \cdot kg^{-1}$, IM; Keta-Control®, Türkiye). Maintenance anesthesia was achieved with isoflurane (Isoflurane®, USA) at a concentration of 2–3%. For analgesia, butorphanol tartrate (0,1 $mg \cdot kg^{-1}$, IM; Butomidor®, Austria) was administered. Following induction, each case was positioned appropriately based on the tumor location, and standard aseptic and antiseptic procedures were applied (FIG. 1).

After isolating the surgical field with sterile drapes, a skin incision was made 3–4 cm away from the visible tumor margins. The incision proceeded through the skin and subcutaneous tissue. If the tumor was invasive to the underlying muscle, the affected muscle tissue was dissected and excised along with the mass. The area was then irrigated with sterile saline, and the incision site was closed using routine surgical suturing techniques. Following surgical excision, the specimens were immersed in 10% neutral buffered formalin and forwarded to the pathology unit for microscopic assessment.

Macroscopic examination

The size, consistency, and color of the excised masses were recorded on gross examiantion.

Histopathological examination

Tissue specimens were fixed in 10% neutral buffered formalin for 48 hours (h). The fixed tissues were trimmed and placed into tissue cassettes. The tissues were processed by Tissue processor (Leica, TP1020, Germany) and embedded in paraffin wax (Leica, HistoCore Arcadia H, Germany). Tissue blocks were sectioned at 5 µm using a microtome (Leica, RM 2125 RT, Germany) and mounted on slides and then stained with Hematoxylin and Eosin and examined under

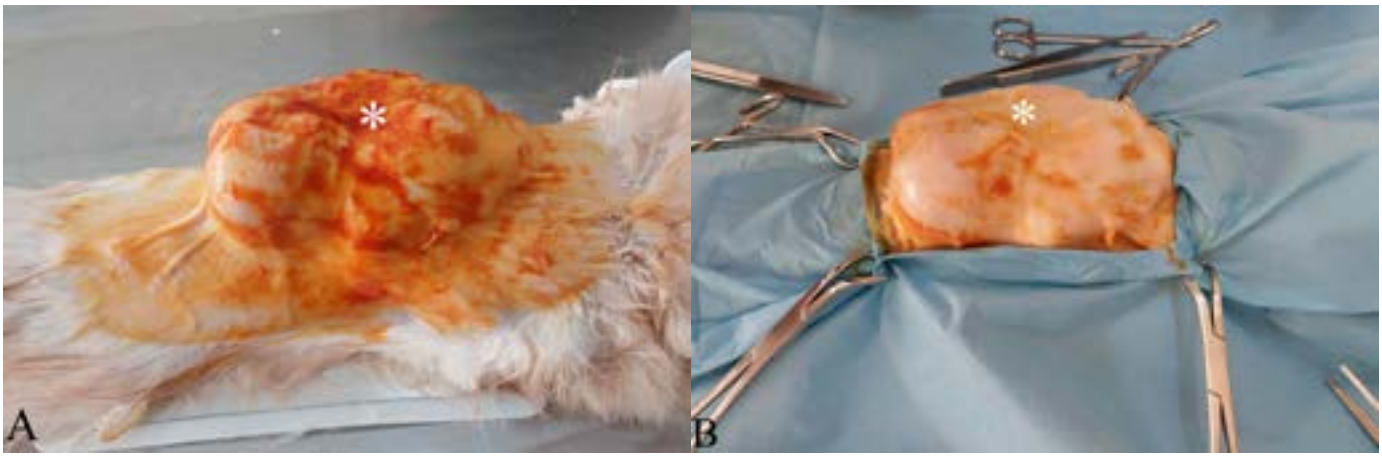

FIGURE 1. Preoperative preparation of the surgical area in a cat with neoplasia. (A) Application of asepsis and antisepsis procedures before excision of the neoplastic tissue. (B) Isolation of the operative field with a sterile drape to maintain aseptic conditions and prevent contamination during tumor removal. * Neoplastic Tissue

a light microscope (Olympus, BX51, Japan) [15]. The tissue origin, biological behavior, degree of cellular atypia, and mitotic index of each neoplastic mass were evaluated and recorded [16]. Microscopic images were captured from selected cases when necessary.

Statistical analysis

Data were loaded on to a database and then subjected to statistical analyses using GraphPad Prism 9.0 (GraphPad Software Inc.®, San Diego, CA, ABD). Data distribution was tested for normality using the Shapiro-Wilk test, while homogeneity of variances was verified with Levene's test. Group comparisons were carried out with an independent samples t-test. Results are expressed as mean ± standard deviation, and differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

In Group T, 57.2% of the subjects were female (n = 4) and 42.8% were male (n = 3). The mean age was 9.71 years with ages of eight years old (4 cats), and twelve years old (3 cats. In Group C the mean age was 2.6 years.

Clinical findings

Physical clinical examination of Group T revealed firm, raised subcutaneous masses in the interscapular region, ranging from 5–6 cm to 10–12 cm in diameter. Of the seven cases, six presented as well-circumscribed masses confined to the subcutaneous tissue. The remaning case exhibited invasive characteristics, with extention beyond subcutaneous tissue (TABLE I).

Preoperative hematological and biochemical profiles revealed no contraindications to surgery. Thoracic radiographs showed preserved anatomy without evidence of metastasis; the trachea maintained normal positioning, pulmonary structures appeared unremarkable, vertebral heart scores were within normal limits, and the diaphragm contour was normal.

Ultrasonographic evaluation revealed distinct morphological patterns correlating with mass size. The three smaller (5–6 cm) masses appeared as heterogeneous hypoechoic nodules with well-defined margins, while the four larger (10–12 cm) masses demonstrated lobulated, compartmentalized architecture with anechoic areas.

Color Doppler imaging identified vascular penetration from periphery to center in all cases. The single case with invasive characteristics, however, exhibited particularly irregular and prominent vascular patterns, corresponding to its infiltrative nature. Surgical exploration confirmed these imaging findings. The three non-infiltrative masses were homogeneous and well-demarcated with minimal vascular invasion, while the larger masses showed irregular borders and prominent vascular connections. The case with invasive characteristics presented unique surgical challenges, as the tumor had invaded the thoracic vertebral spinous processes, necessitating partial resection of affected bone.

TABLE I
Serum Pentraxin–3 levels, clinical, macroscopic and histopathological findings in Feline injection-site sarcomas cases

Case	Serum PTX–3 ($ng \cdot ml^{-1}$)	Clinical finding	Patological finding
1	59.36	Homogeneous, firm consistency, approximately 5–6 cm in size, well-circumscribed	Fibrosarcoma
2	31.18	Homogeneous, firm consistency, approximately 5–6 cm in size, well-circumscribed	Fibrosarcoma
3	89.36	Homogeneous, firm consistency, approximately 5–6 cm in size, well-circumscribed	Fibrosarcoma
4	90.27	Firm consistency, lobulated structure	Pleomorphic Sarcoma
5	354.81	Firm consistency, irregular and lobulated structure, approximately 10–12 cm in size. *Recurrence occurred after 3 months*	Pleomorphic Sarcoma
6	199.36	Firm consistency, irregular and lobulated structure, approximately 10–12 cm in size	Pleomorphic Sarcoma
7	530.27	Invasion into surrounding tissues (proximal parts of the spinous processes of thoracic vertebrae), approximately 15–20 cm in size, lobulated, irregular, and firm consistency. *Recurrence occurred after 3 months*	Pleomorphic Sarcoma

PTX–3: Pentraxin–3, Mean (SD)

Postoperative outcomes differed markedly between cases. The three cases with smaller, non-infiltrative masses remained recurrence-free during follow-up period. Among the four cases with larger masses, two cases developed local recurrence. The case with invasive charecteristic demonstrated aggressive recurrence within three months, ultimately requiring euthanasia (case 7, TABLE I). The remaining case showed no evidence of recurrence during the observation period, highlighting the prognostic significance of initial tumor size and invasiveness observed in this study (TABLE I).

Histopathological findings

Histopathological analysis identified four cases as pleomorphic sarcoma (PS) and three as fibrosarcoma (FS). Among the PS cases, two were classified as storiform-pleomorphic subtype and two as giant cell type. In storiform-pleomorphic subtype, neoplastic areas comprised fibroblast-like and histiocytic cells, accompanied by inflammatory infiltrates (FIGS. 2 A and C). Histiocytic cells were characterized by large, atypical nuclei. The giant cell subtype included flat cells interspersed with histiocytic cells and numerous multinucleated giant cells within the neoplastic zones (FIG. 2D). Both subtypes exhibited frequent mitotic figures. In FS cases, fibroblasts and collagen fibers were interwoven, forming whorled patterns (FIG. 2E). Neoplastic cells were fusiform and of fibrosarcomatous origin, with elongated, oval, and hyperchromatic nuclei (FIG. 2F).

Changes in serum Pentraxin–3

In Group T, serum PTX–3 concentrations ranged from 31.18 to 530.27 ng·mL^{-1}. The first three cases in the TABLE I exhibited low to moderate PTX–3 concentrations (59.36, 31.18, and 89.36 ng·mL^{-1}), had well-circumscribed, homogeneous masses of 5–6 cm, and were diagnosed as fibrosarcoma (TABLE I). Higher PTX–3 values (90.27–530.27 ng·mL^{-1}) were measured generally in cases with larger, irregular, lobulated tumors, all of which were diagnosed as PS (TABLE I). The highest value of PTX3 (530.27 ng·mL^{-1}) was recorded in the case of histopathologically confirmed as PS, which subsequently demonstrated aggressive biological behavior and poor clinical outcomes (case 7, TABLE 1).

As summarised in the TABLE I, cases with serum PTX–3 concentrations above 100 ng·mL^{-1} had larger masses exhibiting irregular contours and a lobulated morphology. In contrast, in cases with tumor diameters < 6 cm and a less aggresive clinical course had serum PTX–3 concentraions within a range comparable to that of the healthy control group (mean ± SD: 45.2 ± 12.8 ng·mL^{-1}).

Comparison of serum PTX–3 levels between Group T (193.52 ± 184.98 ng·ml^{-1}) and Group C (51.62 ± 13.35 ng·ml^{-1}) is shown in FIG. 3. While the mean serum PTX3 concentration was markedly higher in the case group than in the control group, this difference was not statistically significant (P = 0.066).

It is well established that feline injection-site sarcomas develop at sites of vaccination or injection. Consistent with prior reports [6, 8, 17, 18], the tumors in this study were also most frequently localized in the interscapular region. Age distribution of cases was also in line with previous reports [11].

Serum PTX3 concentration in cases was higher though not significant when compared to the control in the present study. Higher concentraions of PTX3 is well documented in neoplasic cases in humans [19, 20, 21].

Studies in human revealed that circulating PTX–3 levels are known to increase with age and correlate with the chronic, low-grade inflammation characteristic of “inflamm-aging” [22, 23]. However, this age-related dynamic has not been directly investigated in veterinary species as there exists no study yet. Consequently, the age difference between the control group and the tumor-bearing cats in this study was not treated as a confounding variable because the diseased cat considerably higher

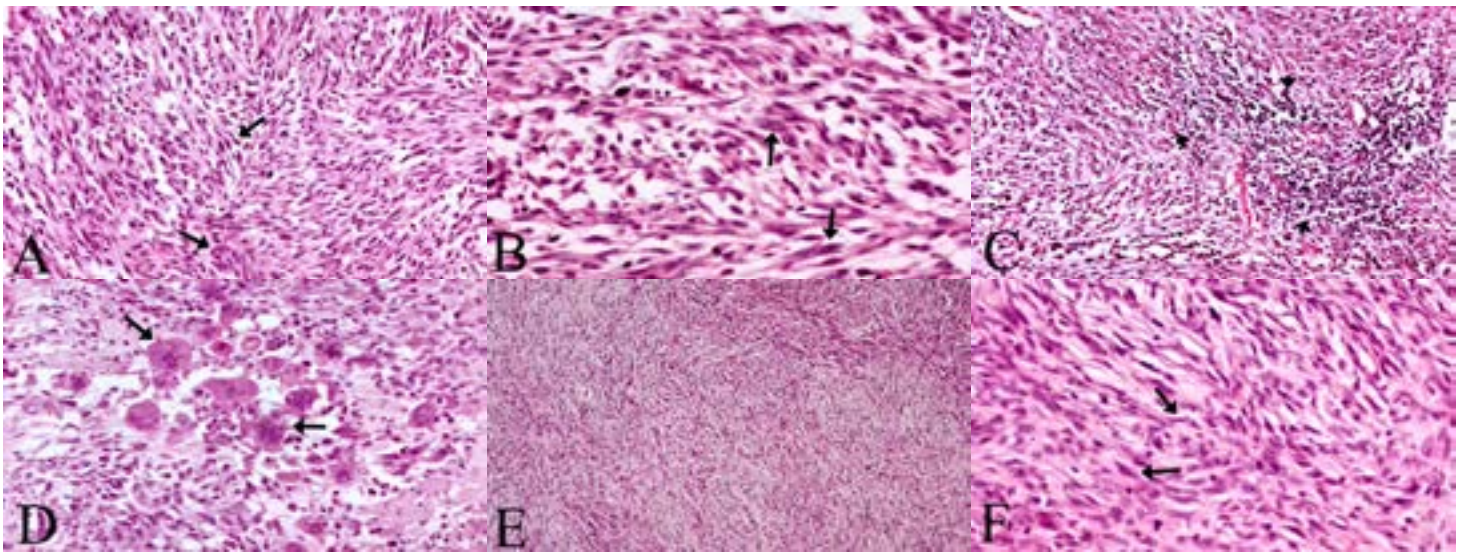

FIGURE 2. A. Pleomorphic sarcoma. Storiform-pleomorphic subtype. Atypical tumour cells (arrows). stained with Hematoxylin and Eosin (HE). 200× magnification. B. Storiform-pleomorphic subtype. Close-up view of atypical tumour cells (arrows). HE. 400× magnification. C. Storiform-pleomorphic subtype. Inflammatory cell infiltrations in the tumour area (arrowheads). HE. 200× magnification. D. Giant cell subtype. Multinucleated giant cells among tumour cells (arrows). 400× magnification. E. Fibrosarcoma. General histological appearance of the tumour. HE. 100× magnification. F. Close-up view of fusiform tumour cells (arrows). HE. 400× magnification

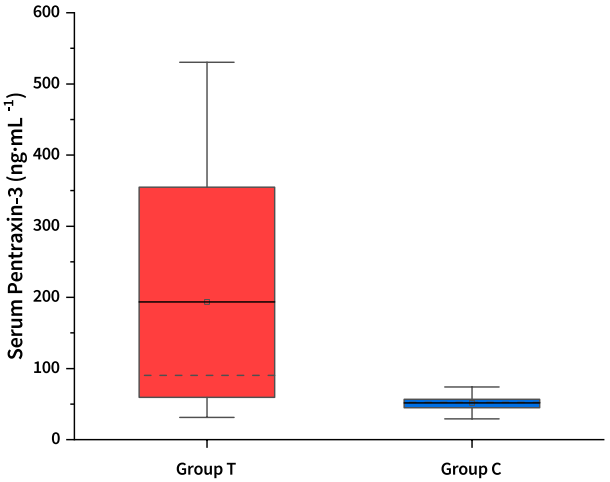

FIGURE 3. Serum pentraxin-3 (PTX3) concentrations (ng·mL^{-1}) in control group (Group C) and cats with neoplasia (Group T)

serum PTX3 that is not explainable by only age but was instead acknowledged as a demographic feature of the cohort.

In Veterinary Medicine, research on PTX–3 has largely centered on infectious diseases and passive immunity transfer [24, 25, 26]. By contrast, its role in veterinary oncology remains poorly explored. This stands in opposition to human oncology, where PTX–3 has established prognostic value across several solid tumors. Elevated PTX–3 levels are linked to advanced disease stage, greater invasiveness, and poorer clinical outcomes in cancers such as colorectal, breast, prostate, and lung carcinoma [4, 14, 19, 20, 27, 28, 29]. This finding that elevated preoperative serum PTX–3 levels correlate with invasive growth and postoperative recurrence in FISS aligns with this human literature. It is important to note, however, that this analysis was limited to a single preoperative measurement; serial postoperative assessments were not performed. Therefore, the prognostic interpretation herein is based on correlating this preoperative baseline with subsequent clinical outcomes.

The mechanistic relevance of PTX–3 in tumor biology is supported by prior research. In colorectal cancer, preoperative serum PTX–3 correlates with invasion depth, lymph node metastasis, and TNM stage, with higher levels predicting reduced survival [27]. In lung cancer models, PTX–3 is implicated in tumor progression, vascularization, and angiogenesis. Both tissue and serum PTX–3 hold prognostic value, and PTX–3 appears to modulate FGF2–mediated angiogenesis, influencing VEGF-related pathways [4, 30, 31]. Collectively, these studies suggest that the elevated PTX–3 levels observed in these cases offer meaningful insight into tumor behavior. This elevation may indicate that PTX–3 expression is stimulated by inflammatory signals within the tumor microenvironment. Thus, in the context of FISS, PTX–3 may serve as a biomarker reflecting underlying tumor aggressiveness.

Finally, key histopathological features-such as inflammatory cell infiltration, osteoclast-like giant cells, and collagen arranged in whorled patterns-were consistent with prior descriptions of FISS [6, 32]. When integrated with elevated serum PTX–3 levels, these morphological findings provide a more comprehensive picture of the tumor's biological activity. Although these findings suggest a potential link between serum PTX–3 levels and the clinical characteristics of FISS, the limited sample size of the present study precludes broader generalization of the findings.

CONCLUSION

PTX–3 is an emerging biomarker of interest in both Veterinary and Human oncology. In inflammation-associated neoplasms such as injection-site sarcomas, assessment of PTX–3 levels may provide supportive information for clinical and pathological evaluation. Although the present study is limited by a small sample size, differences observed in serum PTX–3 levels among histopathological subtypes might suggest a possible association with tumor behavior.

In clinical practice, preoperative measurement of PTX–3, when interpreted together with histopathological findings, may offer additional insight into prognosis. Further studies with larger populations that adjust for several factors such as age, gender, injection site, tumor subtype, are required to clarify the clinical relevance of PTX–3 in feline oncology.

Ethical statement

The study protocol was approved by the Animal Experiments Local Ethics Committee of Aksaray University (Protocol No: 2024/6).

Conflict of interest

The authors declare that they have no competing interests related to the authorship or publication of this article.

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