PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 46:e07799, 2026
DOI: 10.1590/1678-5150-PVB-7799

Original Article
Small Animal Diseases
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Analysis of biomarkers in cats' cavity effusions: differentiating transudates and exudates[1]

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ABSTRACT.- Da Silva RS, Mota JMF, Oliveira A, Silva PHH, Miotto AC, Rossato BG, Moresco RN, Andrade CM. **Analysis of biomarkers in cats' cavity effusions: differentiating transudates and exudates**. *Pesquisa Veterinária Brasileira 46:e07799, 2026.* Universidade Federal de Santa Maria, Av. Roraima 1000, Camobi, Santa Maria, RS 97105-900, Brazil. E-mail: ruschalle@gmail.com

Cavity effusions are common in cats, and differentiating between transudates and exudates is essential for management. This study aimed to evaluate biomarkers in cats' effusions to support differentiation. A total of 44 cats were included, and effusions were classified as transudate (TG) and exudate (EG). The following biomarkers were analyzed: matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO), total protein (TP), glutathione S-transferase (GST), lactate dehydrogenase (LDH), albumin and globulin. For further analysis, effusions were classified as protein-rich transudate (PRT) and EG, and as neoplastic (NEO) or non-neoplastic (nNEO) for MMP-9 and GST evaluation. Non-neoplastic effusions were subdivided into exudates (nnEG) and transudates (nnTG) to assess MPO. MMP-9 was higher in EG ($p = 0.022$), as were MPO ($p = 0.002$), LDH ($p = 0.030$), TP ($p < 0.0001$), albumin ($p = 0.004$), globulin ($p = 0.015$), and cellularity ($p < 0.0001$). GST was higher in TG (0.048). ROC showed discrimination for cellularity (area under the curve – AUC = 0.98, $p < 0.0001$), MPO (AUC = 0.86, $p < 0.0001$), MMP-9 (AUC = 0.73, $p = 0.036$), and LDH (AUC = 0.72, $p = 0.030$). In EG, correlations occurred between MPO and TP ($r = 0.619$, $p = 0.002$), MPO and LDH ($r = 0.624$, $p = 0.012$), and LDH and TP ($r = 0.546$, $p = 0.037$). MPO was higher in PRT ($p = 0.0258$). No differences were observed for MMP-9 and GST between NEO and nNEO, whereas MPO was higher in nnEG ($p = 0.0317$). In conclusion, MPO emerged as a promising biomarker for classifying cats' effusions, particularly in challenging scenarios.

INDEX TERMS: Cavity effusions, biomarkers, myeloperoxidase, matrix metalloproteinase-9.

RESUMO.- [**Análise de biomarcadores em gatos com efusões cavitárias: diferenciação entre transudatos e exsudatos.**] Efusões cavitárias são comuns em gatos, e a diferenciação entre transudatos e exsudatos é essencial para manejo clínico. Este estudo teve como objetivo analisar biomarcadores em efusões de gatos para auxiliar nessa diferenciação. Foram incluídos 44 gatos, cujas efusões foram agrupadas em transudato (TG) e exsudato (EG). Foram analisados, matriz metaloproteinase-9 (MMP-9), mieloperoxidase (MPO), proteína total (TP), glutationa S transferase (GST), lactato desidrogenase (LDH), albumina e globulina. Para análises adicionais, as efusões foram classificadas como transudato rico em proteínas (TRP) e EG, além de neoplásicas (NEO) e não neoplásicas (nNEO) para avaliação de MMP-9 e GST. Efusões não neoplásicas foram subdivididas em exsudatos (nnEG) e transudatos (nnTG) para avaliação da MPO. MMP-9 foi maior no EG ($p = 0.022$), assim como MPO ($p = 0.002$), LDH ($p = 0.030$), TP ($p < 0.0001$), albumina ($p = 0.004$), globulina ($p = 0.015$), e celularidade ($p < 0.0001$). GST foi maior no TG ($p = 0.048$). Análise da curva ROC revelou

[1] Received on December 11, 2025.
Accepted for publication on June 10, 2026.
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poder discriminatória para celularidade (área sob a curva – AUC = 0.98, $p < 0.0001$), MPO (AUC = 0.86, $p < 0.0001$), MMP-9 (AUC = 0.73, $p = 0.036$) e LDH (AUC = 0.72, $p = 0.030$). No EG, observaram-se correlações entre MPO e TP ($r = 0.619$, $p = 0.002$), MPO e LDH ($r = 0.624$, $p = 0.012$) e LDH e TP ($r = 0.546$, $p = 0.037$). MPO foi maior no TRP ($p = 0.0258$). Não foram observadas diferenças para MMP-9 e GST entre NEO e nNEO, enquanto a MPO foi maior em nnEG ($p = 0.0317$, AUC = 0.76, $p = 0.0312$). Em conclusão, a MPO emergiu como um biomarcador promissor para a classificação de efusões em gatos, particularmente em cenários desafiadores.

TERMOS DE INDEXAÇÃO: Efusões cavitárias, biomarcadores, mieloperoxidase, metaloproteinase-9 de matriz.

INTRODUCTION

Cavity effusions are pathological accumulations of fluid within body cavities, classified in veterinary medicine based on cellularity and total protein (TP) into protein-poor transudate (PPT), protein-rich transudate (PRT), and exudate (E) (Valenciano & Rizzi 2020, Boes 2023) Although this approach remains the standard, intermediate or overlapping profiles are frequently observed, making the differentiation between transudates and exudates challenging (Zoia et al. 2009). In such cases, additional biomarkers may enhance diagnostic interpretation and enable a faster classification of effusions.

In human medicine, Light's criteria, based on the ratios of lactate dehydrogenase (LDH) and TP, remain the gold standard for distinguishing transudates from exudates (Light et al. 1972). Simplified versions of these criteria have shown promising results in cats' effusions (Zoia et al. 2009, Smuts et al. 2016, Hazuchova et al. 2023), while the albumin gradient has also demonstrated diagnostic potential (Zoia & Drigo 2016). Recently, biomarkers have emerged as valuable diagnostic tools for effusions. In human medicine, matrix metalloproteinase-9 (MMP-9) is considered a neoplastic biomarker in cavity effusions (Allama et al. 2020). In contrast, in veterinary medicine, it has been reported as an inflammatory biomarker in equine effusions (Hamouzová et al. 2023). Myeloperoxidase (MPO) is recognized as the most effective marker for distinguishing infectious effusions (Alegre et al. 2001). Glutathione-S-transferase (GST) has been shown to exhibit increased expression in neoplastic cells and in cellular injury, although it has not yet been investigated in effusions (Kikuchi et al. 1997). LDH-1 has been investigated as a potential biomarker to distinguish malignant effusions in felines (Naito et al. 2022). Albumin, recognized for its antioxidant properties (Candiano et al. 2009) and role as a negative acute-phase protein, along with TP, remains a valuable parameter for the indirect assessment of inflammation and tissue damage in effusions (Sim et al. 2021).

Given the limited information available for feline effusions, exploring these biomarkers may offer a more refined, rapid, and reliable approach to differentiate transudates from exudates. This study aims to investigate novel biomarkers in cats' cavity effusions to improve the differentiation between transudates and exudates.

MATERIALS AND METHODS

Ethical approval. This study was approved by the Ethics Committee on Animal Use (CEUA) of the "Universidade Federal de Santa Maria" (UFSM), Brazil, under registration number 3928110823.

Cats of various breeds, sexes, and ages, presenting with thoracic, abdominal, and/or pericardial effusions, were selected, and their respective samples were analyzed. To be included in the study, cats needed to present cavity effusion conditioned in a tube containing ethylenediamine tetraacetic acid (EDTA) and in a tube without an additive, and a serum sample. Cats should also have a defined diagnosis for the formation of effusion. Those who were receiving antibiotic therapy or glucocorticoids before effusion collection were excluded. Those with hemorrhagic and chylous cavity effusions were excluded due to their potential interference with the performed analyses.

For the diagnosis of congestive heart failure (CHF), felines were required to present with a compatible clinical history, cardiac alterations detected on auscultation, and confirmation by Doppler echocardiogram. Neoplastic exfoliative effusions (NEO) were diagnosed based on the cytological identification of neoplastic cells morphologically consistent with large cell lymphoma or carcinoma in the effusion samples (Allison 2022) (Fig. 1-2) and confirmed by histopathology. Pyothorax and bacterial/fungal peritonitis were diagnosed with mandatory presence of intracellular bacteria, and a predominance of degenerated neutrophils in effusion (Valenciano & Rizzi 2020, Allison 2022) (Fig. 3). Bacterial cultures were performed, as clinically indicated. When fungal structures were observed, effusions were sent for culture and subjected to polymerase chain reaction (PCR) for identification. Cats with effusions suggestive of feline infectious peritonitis (sFIP) were diagnosed based on a history, clinical signs, compatible clinicopathological changes (lymphopenia and hyperglobulinemia), mandatory presence of intact neutrophils, absence of microorganisms in effusion (Fig. 4), bacterial culture with no growth, effusion TP > 3.5 g/dL, albumin/globulin effusion ratio < 0.45, and positive Rivalta's test (Thayer et al. 2022). In some cases, the diagnosis was further confirmed by histopathological and/or molecular analysis. Acute kidney injury (AKI) was diagnosed in felines presenting with clinical signs, renal azotemia, urinalysis with active sediment (casts and cells) and compatible imaging findings. Hypoproteinemia was defined as serum albumin < 1.5 g/dL, and polycystic liver disease was confirmed through exploratory laparotomy and biopsy. Diagnosis of uroperitoneum (URO) was confirmed when creatinine concentration in effusion was at least twice as high as that in serum (Valenciano & Rizzi 2020).

Cytopathological and biochemical analysis of cavity effusions. EDTA-treated samples were used for cell counting in a hematological apparatus (ABX Micros ES60, Horiba® and Sysmex pocH-100iV Diff®). Cytological evaluation of effusion samples was performed using direct smear (squash technique) and cytocentrifuged preparations (Nova Técnica NT 806, Piracicaba/SP, Brazil). Next, slides were air-dried, stained with Romanosky stain (Quick Panoptic stain, Laborclin-Pinhais/PR, Brazil), and examined under light microscopy at 40x and 100x objectives. A differential cell count was performed in all samples by counting 100 nucleated cells per smear, and both absolute cell counts were recorded.

For biochemical analyses, cavity effusion samples were kept in a tube without additive, whereas serum samples were collected and kept in microtubes. These samples were centrifuged at a force of 900 g for 10 minutes upon arrival at

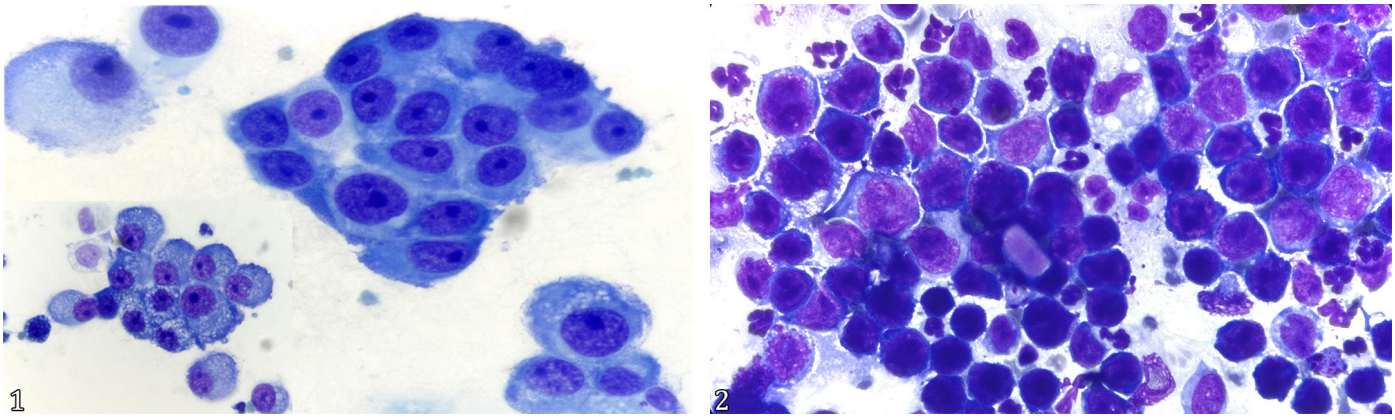


Fig. 1-2. Cytological evaluation of cavity effusions from cats. (**1**) Effusion associated with carcinoma, showing epithelial cells arranged in cohesive clusters, with marked criteria of malignancy, including anisocytosis, anisokaryosis, coarse chromatin, marked cytoplasmic basophilia, cytoplasmic vacuolization, prominent nucleoli, and a moderate nucleus-to-cytoplasm ratio. (**2**) Lymphoma effusion, characterized by a predominant population of large lymphoid cells, with markedly basophilic cytoplasm, occasionally vacuolated, and round to indented nuclei, with coarse chromatin and occasional prominent nucleoli. Quick panoptic stain, oil immersion, obj = 100x.

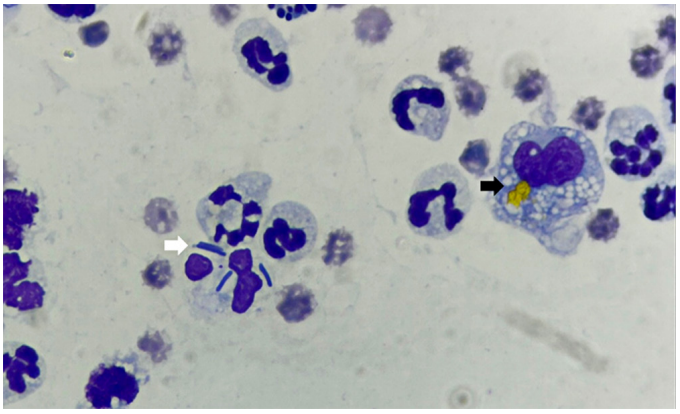


Fig. 3. Cytological evaluation of pleural effusion from a cat with pyothorax. Intracellular bacillary bacteria (white arrow) within a degenerate neutrophil and a hematoidin crystal within a foamy macrophage (black arrow). Quick panoptic stain, oil immersion, obj = 100x.

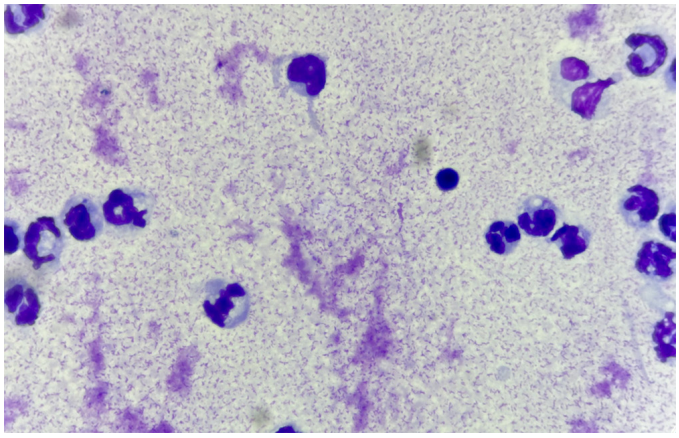


Fig. 4. Cytological evaluation of abdominal effusion suggestive of feline infectious peritonitis. Abundant eosinophilic proteinaceous background, intact neutrophils, and absence of microorganisms. Quick panoptic stain, oil immersion, obj = 100x.

the laboratory, then refrigerated for 24 hours. LDH activity and creatinine concentrations were measured in a BS 120 device (Mindray, Shenzhen, China) using commercial kits (Bioclin®, Belo Horizonte/MG, Brazil). When it was not possible to perform LDH analysis within the aforementioned period, samples were frozen at -20 °C and analyzed within 20 days as recommended by the manufacturer. TP was measured using biuret methodology, and albumin by bromocresol green using commercial kits (Bioclin®, Belo Horizonte/MG, Brazil) on a BS 380 equipment (Mindray, Shenzhen, China). Globulin fraction was obtained by subtracting albumin from the TP, and albumin/globulin ratio (A/G) was also calculated.

Determination of matrix metalloproteinase-9 levels. Cavity effusion and serum samples were aliquoted and stored at -80 °C until analysis with a Cat MMP-9 ELISA Kit (MyBioSource, San Diego, USA). The assay was performed according to the manufacturer's instructions, and absorbance was measured at 450 nm using a microplate photometry equipment (Multiskan FC Thermo Scientific®).

Determination of glutathione-S-transferase activity. Activity was determined as described by Hayes et al. (2005). In a transparent 96-well plate, 20 µL of the sample with 10 µL of reduced L-Glutathione (100 mM) and 240 µL of the system were added. The plate was incubated for two minutes at 37 °C, then the substrate 1-chloro-2,4-dinitrobenzene (CDNB) was added, followed by reading in kinetic mode, wavelength 340 nm, for 30 minutes with 30-second intervals at a temperature of 37 °C. The system used was prepared with 20 mL of potassium phosphate (65.57 mM, pH 7.5), 0.0226 g of EDTA (2.5 mM), and 10.5 mL of distilled H_2O. The results were expressed in µmol CDNB/min/mg of protein. The protein concentration used in the calculation was determined by Bradford (1976).

Determination of myeloperoxidase activity. Activity was measured using a 96-well transparent plate where 15 µL of sample, 140 µL of aminoantipyrine (2.5 mM) and 170 µL of hydrogen peroxide (1.7 mM) were added, followed by plate incubation at 37 °C for 30 minutes and then readings were performed at the endpoint, at a wavelength of 492 nm. Activity was calculated by multiplying the means of the obtained absorbances by a factor of 14, and the results were expressed in µM of quinoneimine produced in 30 minutes.

Classification and composition of groups for biomarker assessment. Effusions were initially classified according to the standard veterinary

method into PPT, PRT, and E, using values proposed by veterinary references (Boes 2023). The effusions were reorganized into two groups for biomarker evaluation: the transudate group (TG), which included both PPT and PRT, and the exudate group (EG). The following biomarkers were evaluated in the cavity effusion sample: MMP-9, LDH, TP, albumin, globulin, A/G ratio, GST, and MPO. An additional analysis was performed to specifically assess the diagnostic performance of biomarkers in the most clinically challenging scenario: differentiating PRT from E. In this analysis, MMP-9, MPO, LDH, and GST levels were compared between the PRT and E groups.

Furthermore, exploratory analyses were performed to investigate the potential role of selected biomarkers in distinguishing neoplastic (NEO) and non-neoplastic (nNEO) effusions. For this purpose, MMP-9 and GST were compared between NEO and nNEO groups. Furthermore, MPO activity was compared between non-neoplastic exudates (nnEG) and non-neoplastic transudates (nnTG) groups.

Statistical analysis. Biomarker data were analyzed using GraphPad Prism version 8.0.1. To compare the TG and EG groups, normality was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Variables with a parametric distribution were analyzed using unpaired t tests, while those with a non-parametric distribution were evaluated, using the Mann-Whitney U test. Effect sizes were calculated for all between-group comparisons using Cohen's d, considering differences as EG minus TG, and were interpreted as small (0.2), medium (0.5), or large (0.8). Receiver operating characteristic (ROC) curve analysis was performed to determine diagnostic cutoff values for each biomarker. Optimal cutoff points were selected based on the maximum Youden index (J), defined as sensitivity + specificity − 1, providing the best balance between sensitivity and specificity for the study population. Additionally, correlations between selected biomarkers were evaluated using Pearson or Spearman correlation coefficients, depending on data distribution. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Animals

This prospective study included 44 cats with cavity effusions. The mean age was 58.4 months (SD: 43.60 months; range 3–168 months). The mean age of cats in the TG was 80 months, compared to 43.4 months in the EG, representing a statistically significant difference (p = 0.0221). In this study, 54.5% (24/44) of the cats were female, and 45.5% (20/44) were male. Effusions were divided into TG (n = 18) and EG (n = 26) to analyze diagnostic biomarkers. Regarding effusion location, 59.1% (26/44) were thoracic, 38.6% (17/44) abdominal, and 2.3% (1/44) pericardial. In the TG group, the underlying diagnoses included NEO 33.4% (6/18), IKA 22.2% (4/18), CHF 22.2% (4/18), URO 11.1% (2/18), and hypoproteinemia 11.1% (2/18). In the EG, NEO 50% (13/26), bacterial peritonitis 15.4% (4/26), bacterial pleuritis 7.7% (2/26), sPIF 15.4% (4/26), CHF 3.8% (1/26), URO 3.8% (1/26) and polycystic liver disease 3.8% (1/26). For the targeted analysis of PRT and E differentiation, a PRT subgroup (n = 11) was compared with E (n = 26). A secondary classification categorized effusions as NEO (n = 19) or nNEO (n = 25). Additionally, non-neoplastic effusions were further subdivided into nnEG (n = 13) and nnTG (n = 12).

Evaluation of biomarkers of cavity effusions

Biomarker analyses in cavity effusions revealed significant differences between the EG and TG groups. Exudates showed significantly higher values of MMP-9 (p = 0.0222), LDH (p = 0.0307), TP (p < 0.0001), albumin (p = 0.0043), globulin (p = 0.0153), TNCC (p < 0.0001), and MPO (p = 0.0020) compared to transudates. In contrast, GST was significantly associated with transudates (p = 0.0485), as shown in Figure 5 and Table 1. ROC curve analyses demonstrated that TNCC showed the highest diagnostic performance for differentiating exudates from transudates (area under the curve – AUC = 0.98), followed by MPO (AUC = 0.86) and TP (AUC = 0.85). Other biomarkers showed moderate accuracy, including MMP-9 (AUC = 0.73), LDH (AUC = 0.72), albumin (AUC = 0.77), and globulin (AUC = 0.72), while GST showed lower diagnostic performance (AUC = 0.67), as shown in Figure 6 and Table 2. In the EG, moderate positive correlations were observed between MPO and TP (r = 0.619, p = 0.002), MPO and LDH (r = 0.624, p = 0.012), and LDH and TP (r = 0.546, p = 0.037), as presented in Table 3. In the TG, a moderate negative correlation was observed between TP and GST (r = -0.559, p = 0.024), as detailed in Table 4. All statistically significant correlations are illustrated in Figure 7. In the targeted analysis comparing PRT and E, MPO was the only biomarker

Table 1. Mean, standard deviation, and p-values of biomarkers measured in cavity effusions from 44 cats

	TG (n = 18)	EG (n = 26)	p-value	Effect size (Cohen's d)
MMP-9 (ng/mL)	30.44 ± 10.12	39.56 ± 8.77	0.0222	0.96
LDH (U/L)	149.7 ± 149.0	511.68 ± 468.03	0.0307	0.95
TP (g/dL)	2.69 ± 1.57	5.08 ± 1.69	< 0.0001	1.46
Albumin (g/dL)	1.0 ± 0.61	1.73 ± 0.79	0.0043	1.02
Globulin (g/dL)	1.67 ± 1.24	2.83 ± 1.47	0.0153	0.86
A/G	0.71 ± 0.09	0.64 ± 0.21	0.1658	-0.41
GST (µmol CDNB/min/mg of protein)	91.06 ± 30.47	64.30 ± 24.87	0.0485	-0.96
TNCC (/mm^3)	1.119 ± 1.312	27.386 ± 28.937	< 0.0001	1.28
MPO (µM)	1.15 ± 0.58	5.24 ± 4.27	0.0020	1.33
	PRT (n = 11)	E (n = 26)	p	Effect size (Cohen's d)
MPO (µM)	1.52 ± 0.7	3.91 ± 3.48	0.0258	- 0.80
MMP-9 (ng/mL)	36.74 ± 15.2	38.47 ± 9.9	0.6836	- 0.15
LDH (U/L)	291.3 ± 219.6	565.7 ± 571.9	0.4017	- 0.55
GST (µmol CDNB/min/mg of protein)	71.21 ± 35.22	69.46 ± 30.33	0.7749	0.06

TG = Transudate group, EG = exudate group, MMP-9 = matrix metalloproteinase-9, LDH = lactate dehydrogenase, TP = total protein, A/G = albumin/globulin ratio, GST = glutathione-S-transferase, CDNB = 1-chloro-2,4-dinitrobenzene, TNCC = total nucleated cell count, MPO = myeloperoxidase, PRT = protein-rich transudate, E = exudate.

that showed a statistically significant difference between groups (p = 0.0258), while MMP-9 (p = 0.6836), LDH (p = 0.4017), and GST (p = 0.7749) were not significantly different, as shown in Figure 8 and Table 1. ROC analysis demonstrated a moderate ability of MPO to discriminate between PRT and E (AUC = 0.76), although statistical significance was not reached (p = 0.08), in Table 2. No statistically significant differences were observed for MMP-9 and GST between the NEO and nNEO groups (p = 0.556 and p = 0.1097, respectively) (Table 5). In contrast, MPO showed a statistically significant difference, with higher ativity in the nnEG group compared to the nnTG group (p = 0.0317), as shown in Figure 8 and Table 5. ROC analysis demonstrated a moderate ability of MPO to discriminate between nnEG and nnTG (AUC = 0.76, p = 0.0312), as shown in Figure 9 and Table 2.

DISCUSSION

The significant age difference between groups likely reflects the underlying diseases that cause transudative and exudative effusions in cats. Older animals tend to develop effusions secondary to chronic or degenerative conditions, while younger cats are more often affected by acute or inflammatory processes. This pattern has been described in previous veterinary studies, where age was associated with both the type and chronicity of effusion (Naito et al. 2022). Therefore, age, although not a diagnostic criterion by itself, provides valuable clinical context that can guide the interpretation of effusion characteristics and potential etiologies.

Our findings highlight several biomarkers, particularly cellularity, MMP-9, MPO and LDH, that were predominantly elevated in exudative samples and demonstrated good diagnostic accuracy in the ROC curve analysis for distinguishing exudates from transudates. These results reflect their close association with inflammatory, infectious, or neoplastic processes and reinforce their potential as complementary diagnostic tools.

Among these, MMP-9 emerges as a novel marker for cats' effusions. It has been previously studied in human and equine effusions,

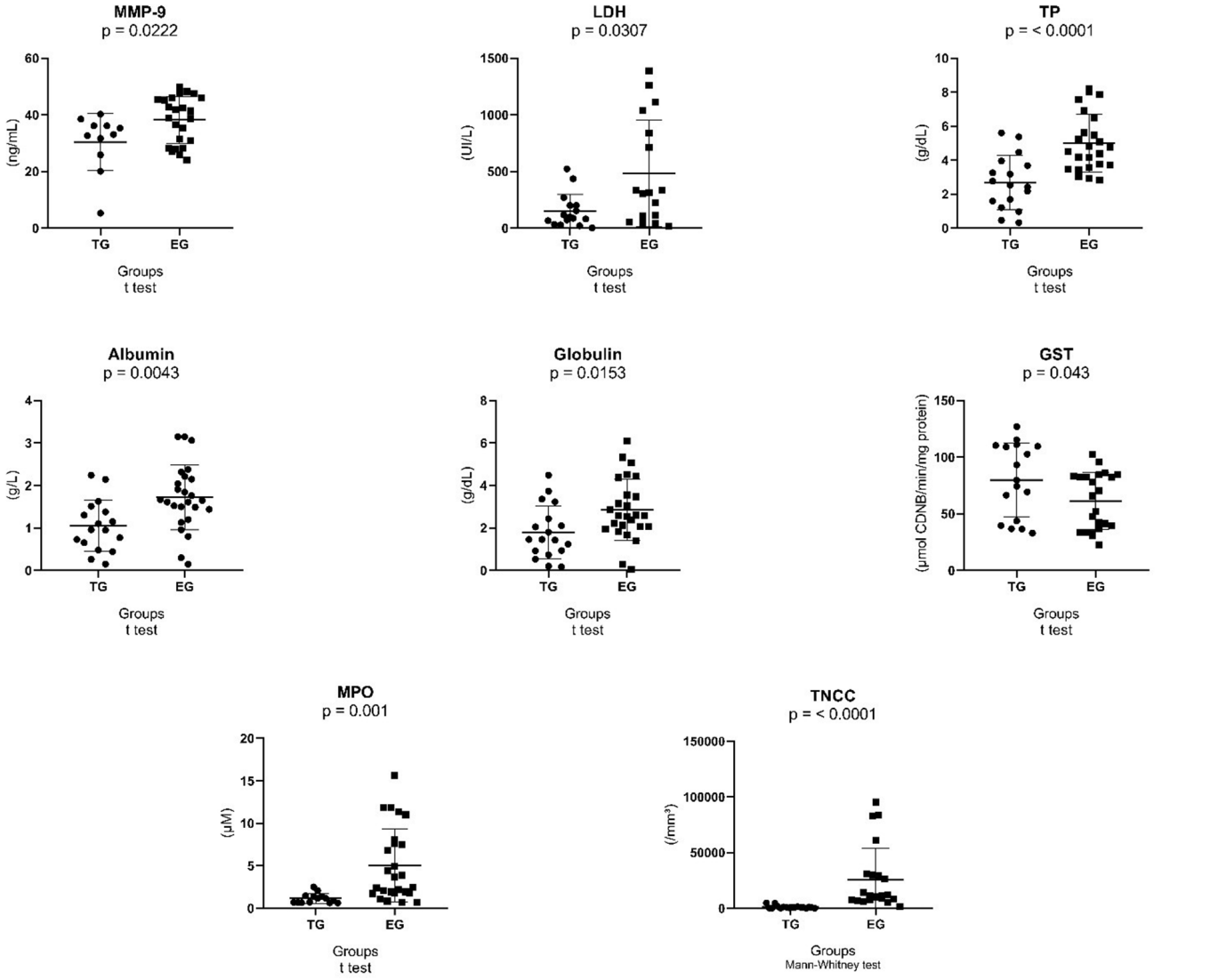


Fig. 5. Graph showing the statistically significant differences in biomarker analysis of cavity effusions from 44 cats, comparing the transudate (TG) and exudate (EG) groups. The evaluated parameters include matrix metalloproteinase-9 (MMP-9), lactate dehydrogenase (LDH), total protein (TP), albumin, globulin, glutathione S-transferase (GST), myeloperoxidase (MPO), and total nucleated cell count (TNCC). p considered significant when < 0.05.

Table 2. Cut-off values, sensitivity, specificity, accuracy, and receiver operating characteristic (ROC) curve parameters for biomarkers in differentiating exudative (EG) from transudative (TG) effusions in cats. Additionally, ROC analysis for myeloperoxidase (MPO) was performed to differentiate non-neoplastic exudates (nnEG) from non-neoplastic transudates (nnTG)

Marker	Cut-off value	Sensitivity (%)	Specificity (%)	Accuracy (%) (CI 95%)	AUC (CI 95%)	*p*-value*
MMP-9 (ng/mL)	> 40.8	50.00	100	70.45 (55.2 – 82.5)	0.73	0.036
LDH (U/L)	> 286.9	59.00	87.5	70.45 (55.2 – 82.5)	0.72	0.030
TP (g/dL)	> 4.48	54.17	88.24	68.18 (53 – 80.4)	0.85	0.000
Albumin (g/dL)	> 1.64	52.00	88.24	67.27 (53 – 80.4)	0.77	0.003
Globulin (g/dL)	> 2.11	69.23	72.22	70.45 (55.2 – 82.5)	0.72	0.011
GST (µmol CDNB/min/mg of protein)	> 66.16	52.17	70.59	59.10 (44.2 – 72.6)	0.67	0.068
TNCC (/mm^3)	> 5.150	95.45	100	97.73 (88.2 – 99.6)	0.98	< 0.0001
MPO (µM)	> 2.14	61.54	92.86	75.0 (60 – 85.9)	0.86	0.000
MPO (µM) – PRT vs E	> 2.14	58.33	87.50	67.6 (51.5 – 80.4)	0.76	0.0873
MPO (µM) – nnEG vs nnTG	> 1.68	81.82	75.0	80.0 (60.9 – 91.1)	0.76	0.0312

CI = Confidence interval, AUC = area under the ROC curve, MMP-9 = matrix metalloproteinase-9, LDH = lactate dehydrogenase, TP = total protein, GST = glutathione-S-transferase, TNCC = total nucleated cell count, PRT = protein-rich transudate, E = exudate; * *p* considered significant when < 0.05.

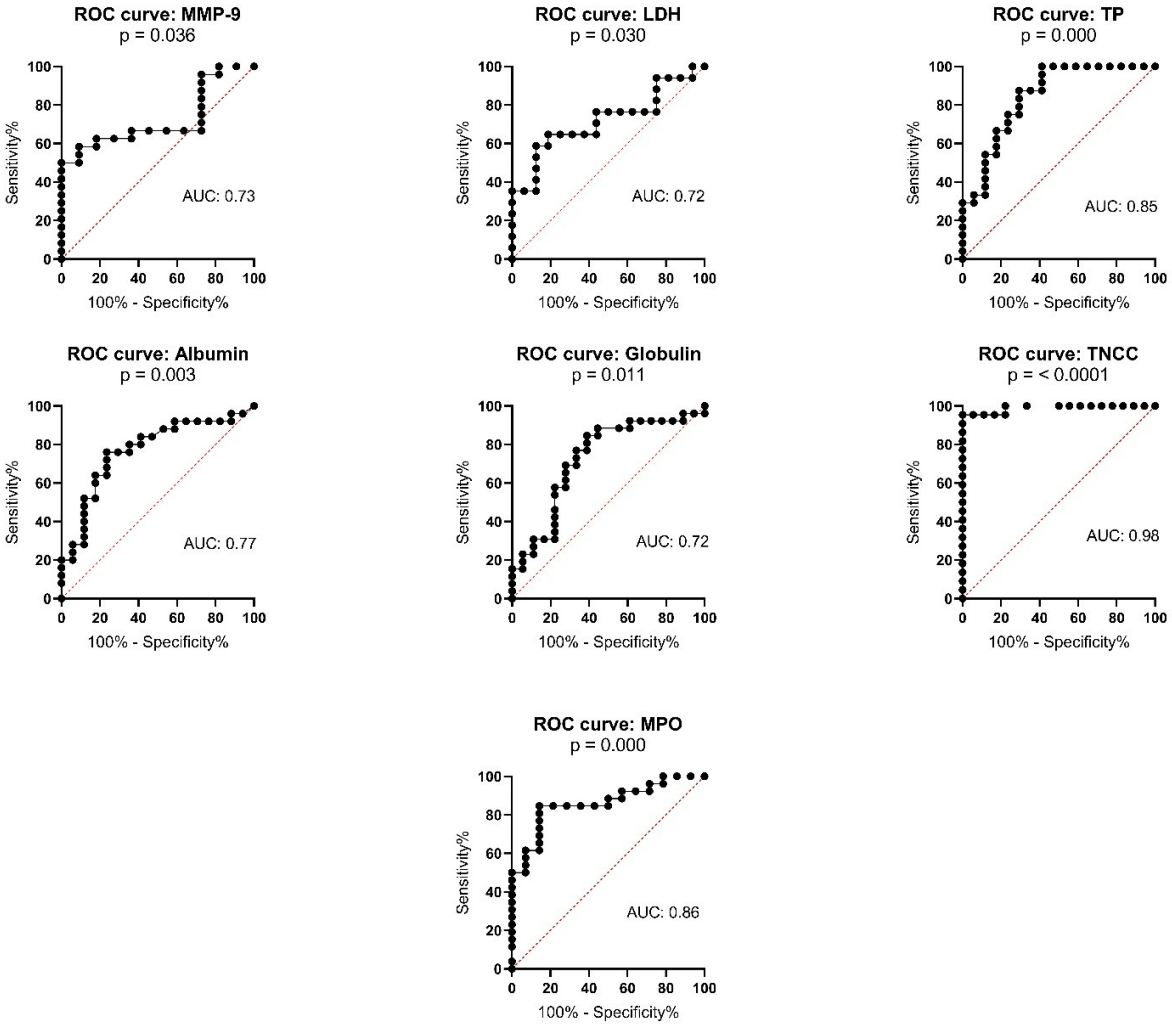


Fig. 6. Receiver operating characteristic (ROC) curves for the evaluated biomarkers to discriminate between exudates (EG) and transudates (TG) in cats' cavity effusions. ROC curves displaying the diagnostic performance of matrix metalloproteinase-9 (MMP-9), lactate dehydrogenase (LDH), total protein (TP), albumin, globulin, total nucleated cell count (TNCC), and myeloperoxidase (MPO). For each biomarker, the *p*-value indicates the statistical significance of the model, and the area under the curve (AUC) reflects its diagnostic accuracy, with higher AUC values indicating superior discriminative ability. Black dots denote the optimal cutoff points based on the best balance between sensitivity and specificity, and the red dashed line represents the reference line corresponding to an AUC of 0.5.

where it was identified as a marker for neoplastic and inflammatory processes, respectively (Allama et al. 2020, Hamouzová et al. 2023). In our study, the observed increase in MMP-9 in the EG group likely reflects enhanced local inflammatory activity (Hamouzová et al. 2023). Elevated MMP-9 may be linked to inflammation, neoplasia, or chronic infections, in which sustained pro-inflammatory cytokine release is known to induce MMP-9 production (Lee & Kim 2022). However, no statistically significant differences in MMP-9 levels were observed between neoplastic and non-neoplastic effusions, indicating that this biomarker may not be a reliable indicator of neoplastic status in cats. Correlation analysis did not reveal significant associations between MMP-9 levels and the absolute counts of neutrophils, lymphocytes, or macrophages, nor with the TNCC, suggesting that MMP-9 is not directly related to cellularity. Rather, it appears to

Table 3. Correlations of biomarkers in exudative effusions cavity in 44 cats

Biomarkers in exudates	r	*p*-value*
MMP-9 vs. MPO	0.108	0.625
MMP-9 vs. TNCC	- 0.132	0.578
MPO vs. TNCC	0.133	0.556
MPO vs. TP	0.619	0.002
MMP-9 vs. TP	0.179	0.414
MPO vs. LDH	0.624	0.012
LDH vs. TNCC	0.154	0.584
MMP-9 vs. LDH	0.107	0.705
LDH vs. albumin	0.491	0.054
LDH vs. TP	0.546	0.037
MMP-9 vs. neutrophils	0.444	0.065
MMP-9 vs. lymphocytes	0.342	0.165
MMP-9 vs. macrophages	- 0.201	0.423
MPO vs. neutrophils	0.155	0.539
MPO vs. lymphocytes	- 0.352	0.152
MPO vs. macrophage**s**	- 0.141	0.577
LDH vs. neutrophils	0.225	0.416
LDH vs. lymphocytes	- 0.216	0.436
LDH vs. macrophage**s**	0.254	0.359
TP vs. neutrophils	- 0.049	0.852
TP vs. lymphocytes	0.223	0.386
TP vs. macrophages	- 0.065	0.802

MMP-9 = matrix metalloproteinase-9, MPO = myeloperoxidase, TNCC = total nucleated cell count, TP = total protein, LDH = lactate dehydrogenase; * *p* considered significant when < 0.05.

Table 4. Correlations of biomarkers in transudative

Biomarkers in transudates	r	*p*-value
GST vs. TP	- 0.559	0.024
GST vs. TNCC	- 0.435	0.081
GST vs. albumin	- 0.345	0.191
GST vs. globulin	- 0.310	0.226
GST vs. neutrophils	- 0.058	0.853
GST vs. lymphocytes	- 0.154	0.613
GST vs. macrophages	0.157	0.563

Glutathione-S-transferase, TP = total protein, TNCC = total nucleated cell count; * *p* considered significant when < 0.05.

Table 5. Exploratory analysis of biomarkers in cats' cavity effusions according to neoplastic status

	NEO (n=19)	nNEO (n=25)	*p*-value*	Effect size (Cohen's d)
MMP-9 (ng/mL)	39.29 ± 10.47	37.28 ± 12.31	0.556	0.18
GST (µmol CDNB/min/mg of protein)	62.95 ± 30.21	79.65 ± 33.04	0.1097	-0.53
	nnEG (n=13)	nnTG (n=12)	*p*-value*	Effect size (Cohen's d)
MPO (µM)	6.21 ± 5.06	2.29 ± 2.76	0.0317	0.94

NEO = Neoplastic group, nNEO = non-neoplastic group, MMP-9 = matrix metalloproteinase-9, GST = glutathione-S-transferase, CDNB = 1-chloro-2,4-dinitrobenzene, nnEG = non-neoplastic exudate group, nnTG = non-neoplastic transudate group, MPO = myeloperoxidase; * *p* considered significant when < 0.05.

reflect functional cellular activation and local tissue remodeling within the effusion microenvironment. Therefore, the observed increase in MMP-9 supports its potential role as a biomarker for differentiating exudative effusions in cats, rather than as a specific indicator of tumor infiltration.

MPO has been identified as a promising new biomarker in cats' exudative effusions, showing strong discriminatory capacity between groups, which aligns with its role as a neutrophil-derived enzyme involved in the acute inflammatory response (Valadez-Cosmes et al. 2022). In human medicine, pleural fluid MPO is considered one of the best biomarkers for characterizing effusions associated with infectious processes (Alegre et al. 2001), which reinforces this finding in the feline species. Positive correlations between MPO and TP, as well as between MPO and LDH, suggest that inflammation and cell lysis occur interdependently in exudative effusions. The concomitant elevation of LDH in the EG group confirms this relationship, as this enzyme is released following cellular membrane damage, reflecting necrosis or tissue degradation (Dawson 1964). When the analysis was restricted to non-neoplastic effusions, MPO levels remained significantly higher in exudates than in transudates, confirming that inflammatory processes, rather than the presence of neoplasia, drive this difference. ROC curve analysis further supported its discriminatory capacity, demonstrating a statistically significant performance. It is important to highlight that, when analyzing the most diagnostically challenging scenario, namely the differentiation between PRT and exudates, MPO was the only biomarker that remained significantly different between groups, whereas MMP-9, LDH, and GST did not demonstrate statistical differences. The ability of MPO to differentiate these groups likely reflects its association with neutrophil activation and inflammatory activity. Although ROC curve analysis demonstrated a moderate discriminatory performance for MPO in this context, statistical significance was not achieved, likely due to the limited sample size of the PRT group and the intrinsic variability and partial overlap between groups. Nevertheless, the magnitude of the observed effect and the significant difference in MPO levels support its potential clinical relevance in this specific

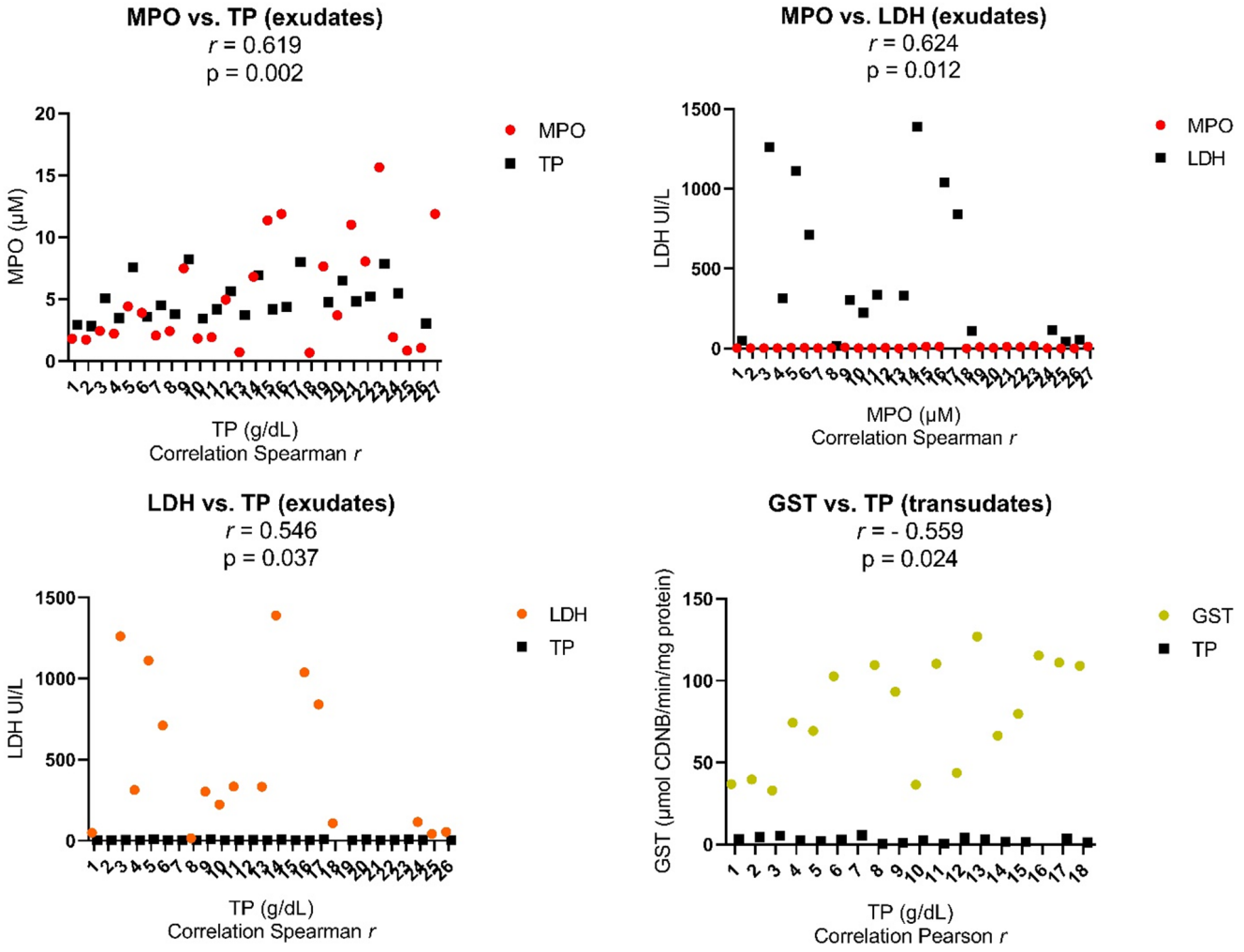


Fig. 7. Correlations between the biomarkers myeloperoxidase and total protein (MPO vs. TP), myeloperoxidase and lactate dehydrogenase (MPO vs. LDH), and lactate dehydrogenase and total protein (LDH vs. TP) in exudative effusions; and glutathione S-transferase and total protein (GST vs. TP) in transudative effusions of cats with cavity effusions.

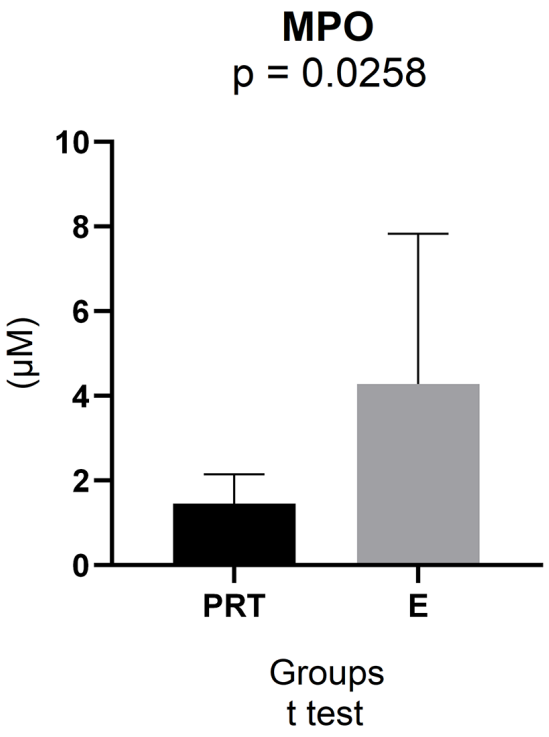


Fig. 8. Graph showing the statistically significant difference in myeloperoxidase (MPO) activity in cavity effusions from 37 cats, comparing the protein-rich transudate (PRT) and exudate (E) groups. *p* was considered significant when < 0.05.

scenario. To the best of our knowledge, this is the first study to evaluate MPO activity in cavity effusions in veterinary medicine.

The increased concentrations of albumin and globulin observed in exudative effusions are consistent with the classical criteria used to differentiate cavity effusions, in which enhanced vascular permeability and leakage of plasma proteins are characteristic features of the inflammatory process (Boes 2023). These findings support the interpretation that the mechanisms underlying exudative effusion formation involve both protein leakage and immune activation, complementing the biochemical results observed in this study.

GST showed higher activity in the TG, although transudates are generally associated with hemodynamic alterations rather than active cellular processes. This increase represents a novel observation in transudative cavity effusions, which has not yet been investigated in detail. It may reflect local mechanisms of response to oxidative stress, as described in other tissues (Mazari et al. 2023). Consistent with this, GST showed a moderate negative correlation with TP and weak, non-significant correlations with TNCC, albumin, and globulin, suggesting that its activity is largely independent of fluid cellularity or protein content. Despite its elevation in transudates, GST did not demonstrate good accuracy on ROC analysis, indicating limited diagnostic utility. In addition, GST did not show a consistent association with neoplastic effusions, indicating that this enzyme was not a reliable neoplastic biomarker in cats' cavity effusions.

CONCLUSION

The analysis of biomarkers in cats' cavity effusions proved to be a promising approach for improving the differentiation between transudative and exudative effusions. Among the evaluated parameters,

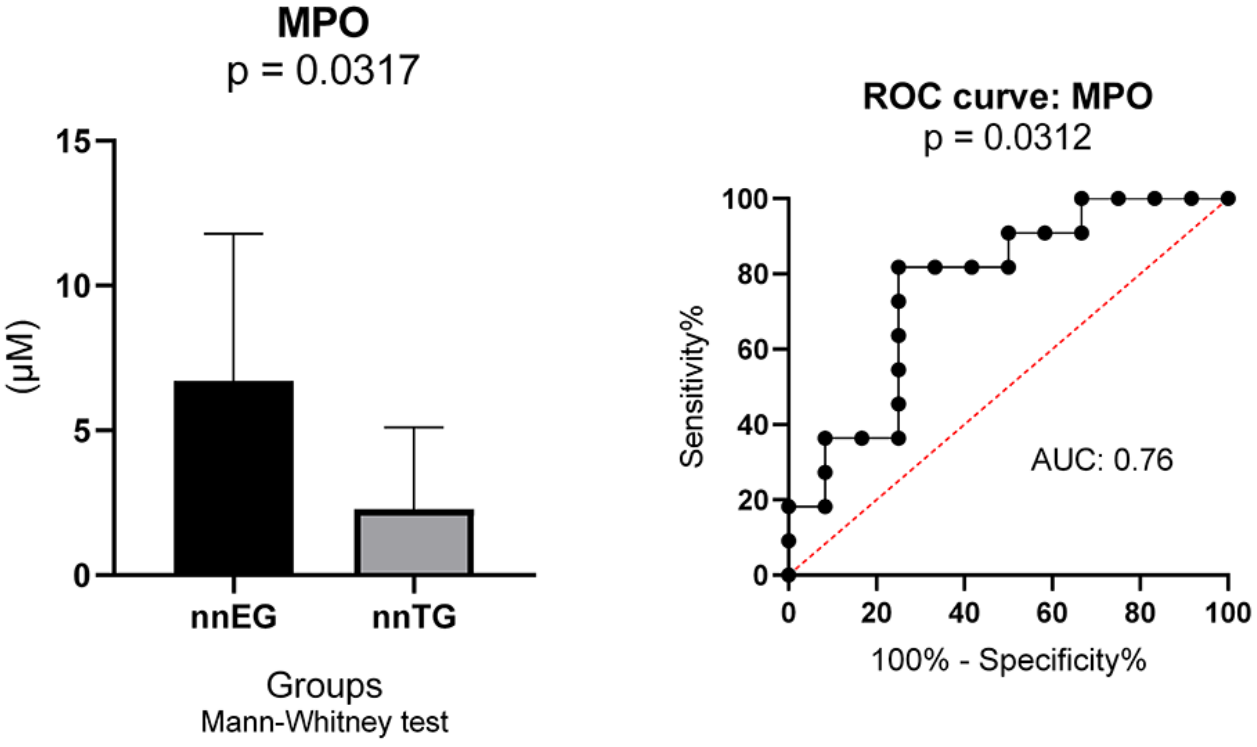


Fig. 9. Myeloperoxidase (MPO) analysis in cavity effusions from 25 cats. The left graph shows the comparison between non-neoplastic exudates (nnEG) and non-neoplastic transudates (nnTG), demonstrating a statistically significant difference ($p = 0.0317$; Mann-Whitney test). The right graph presents the receiver operating characteristic (ROC) curve for MPO (area under the curve – AUC = 0.76, $p = 0.0312$). Black dots represent the evaluated cutoff points, and the red dashed line indicates the reference line (AUC = 0.5).

matrix metalloproteinase-9 (MMP-9), lactate dehydrogenase (LDH), cellularity, and, in particular, myeloperoxidase (MPO) demonstrated diagnostic value. MPO showed the most consistent performance, including in clinically challenging scenarios such as differentiating protein-rich transudates from exudates, supporting its potential as a complementary biomarker for classifying cats' effusions. Overall, these findings support the integration of biochemical biomarkers into conventional diagnostic approaches and contribute to a better understanding of the pathophysiology of cats' effusion.

Acknowledgments.- This study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brazil – Finance Code 001, and by "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq).

Conflict of interest statement.- The authors declare that they have no conflicts of interest.

Credit author statement.- Rúbia S. Silva: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – original draft, Writing – Review & editing, Visualization. Jéssica M.F. Mota: Data curation, Investigation, Formal analysis. Augusto de Oliveira: Investigation, Data curation. Paulo H.H. Silva: Data curation, Software, Validation, Writing – Review & editing. Anna C. Miotto: Resources, Data curation. Bruna G. Rossato: Data curation, Investigation. Rafael N. Moresco: Data curation, Validation. Cinthia M. Andrade: Writing – Review & editing, Conceptualization, Validation, Supervision.

Data availability statement.- The data used in this study are available and can be accessed upon request to the corresponding author.

Editor-in-Chief.- Fabiano José Ferreira de Sant'Ana.

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