

Evaluation of tumor markers carcinoembryonic antigen, cytokeratin 19 fragment and cancer-associated antigen 72-4 in neoplastic and non-neoplastic canine effusions differentiation

[Avaliação dos marcadores tumorais antígeno carcinoembrionário, fragmento de citoqueratina 19 e antígeno associado ao câncer 72-4 na diferenciação de efusões neoplásicas e não neoplásicas caninas]

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ABSTRACT

The concentration of tumor markers in body fluids can be used for diagnosis and prognosis of patients. This study aimed to investigate the performance of tumor markers cytokeratin 19 fragment (CYFRA 21-1), cancer-associated antigen 72-4 (CA 72-4) and carcinoembryonic antigen (CEA) in the neoplastic and non-neoplastic canine effusions. In thirty-two neoplastic (n=16) and non-neoplastic (n=16) samples of canine thoracic or abdominal effusions, tumor markers were measured. Significant statistical difference was found only for the CYFRA 21-1 marker. The levels were significantly higher for the neoplastic group. The lack of significance between groups for markers CA 72-4 and CEA can be explained by the presence of other diseases in the non-neoplastic group, causing elevated levels of these markers. This study concludes that CYFRA 21-1 performed well, showing good sensitivity, specificity and accuracy in the diagnosis of neoplastic effusions in dogs. However, further investigations are necessary in patients with malignancy as those with benign effusions.

Keywords: dog, body cavity fluid, CEA, CA 72-4, CYFRA 21-1

RESUMO

Os níveis de marcadores tumorais em líquidos corporais podem ser usados para diagnóstico e prognóstico de pacientes. Este estudo objetiva investigar o desempenho dos marcadores tumorais fragmento de citoqueratina 19 (CYFRA 21-1), antígeno associado ao câncer 72-4 (CA 72-4) e antígeno carcinoembrionário (CEA) em efusões caninas neoplásicas e não neoplásicas. Os marcadores tumorais foram mensurados em 32 amostras de efusões torácicas e abdominais de cães, 16 neoplásicas e 16 não neoplásicas. Foi encontrada diferença estatística somente para o marcador CYFRA 21-1, onde os níveis foram significativamente altos no grupo neoplásico. A falta de significância entre os grupos de marcadores CA 72-4 e CEA pode ser explicada pela presença de outras doenças no grupo não neoplásico, o que causou elevação dos níveis destes marcadores. Este estudo conclui que o marcador CYFRA 21-1 teve bom desempenho, pois mostrou boa sensibilidade, especificidade e acurácia no diagnóstico de efusões neoplásicas em cães. Entretanto, mais estudos são necessários tanto em pacientes portadores de efusões benignas quanto malignas.

Palavras-chave: cão, líquido cavitário, CEA, CA 72-4, CYFRA 21-1

INTRODUCTION

Neoplasia is a common cause of effusions in dogs. O'Brien and Lumsden (1988) reported that 57% of pericardial effusions and 11% of peritoneal and pleural effusions result from

tumor processes in this species. However, neoplastic cells are not always present within the effusion, or are sometimes visible in small amounts, hindering cytological diagnostic (Clinkenbeard, 1992; Edwards, 1996).

Cytological evaluation of effusions is one of the main diagnostic methods for detecting neoplasia, although it shows a sensitivity of 60% (Fenton and Richardson, 1995; Light, 2001). Therefore, the role of biochemical tumor markers in the characterization of neoplasia is widely investigated in human medicine (Romero *et al.*, 1996; Ferrer *et al.*, 1999; Gross, 1999; Miédougé *et al.*, 1999; Ferrer, 2000; Alatas *et al.*, 2001; Buccheri and Ferrigno, 2001; Villena *et al.*, 2003; Trapé *et al.*, 2004) and studies on its usefulness in veterinary medicine have increased in recent years (Lowseth *et al.*, 1991; Hahn and Richardson, 1995; Lechowski *et al.*, 2002; Kumar and Pawayia, 2010).

To better identify malignant effusions, many studies have reported the use of biochemical tumor markers (Ferrer, 2000; Buccheri and Ferrigno, 2001). These markers are defined as substances produced by the neoplastic cells or by the host in response to the presence of the tumor that can be detected in body fluids and used in the managing of cancer patients (Chan and Schwartz, 2002; Trapé *et al.*, 2011).

The cytokeratin 19 fragment (CYFRA 21-1) presents good sensitivity for squamous cell carcinoma of the lungs and mesothelial cells, although it may also be present in the normal epithelium. This can generate false positive results in some benign diseases, such as gastrointestinal, urological or gynecological inflammatory processes. The cancer-associated antigen 72-4 (CA 72-4) is sensitive for a variety of adenocarcinomas, especially in the gastric region. The carcinoembryonic antigen (CEA) presents high levels particularly in neoplasms of epithelial origin. Many studies showed that the association between different tumor markers increases the sensitivity for the diagnosis of malignant effusions (Romero *et al.*, 1996; Ferrer, 2000; Villena *et al.*, 1996; Dejsomritrutai *et al.*, 2001). The goal of this study was to investigate the performance of the biochemical tumor markers CYFRA 21-1, CA 72-4 and CEA in differentiating between neoplastic and non-neoplastic effusions in dogs.

MATERIAL AND METHODS

For eight months, a total of 59 samples of canine thoracic and abdominal effusions from animals referred to the Veterinary Clinical Hospital of

Federal University of Rio Grande do Sul were collected following technical procedures according to Kruth (2004). Samples were tapped into dry plastic tubes with anticoagulant (ethylenediaminetetraacetic acid) and analyzed immediately. The study was accepted by the Ethic Commission for the Use of Animals (CEUA) of the Federal University of Rio Grande do Sul (project number 19.641).

All samples were analyzed physically, biochemically and cytologically according to Raskin and Meyer (2001), Bibbo and Longato Filho (2007), and Cowell *et al.* (2009). Samples were divided into two groups according to cytological characteristics: neoplastic and non-neoplastic. The effusions were classified as neoplastic when cells exhibiting at least three criteria for malignancy were appreciated in the cytological assessment. For a complete diagnosis medical records and imaging examinations were also considered for each patient. After examination, the supernatant was fractioned in aliquots and quickly stored at -80°C for posterior determination of biochemical tumor markers.

Of the 59 samples analyzed, 32 were selected for determination of tumor markers. The remaining 27 were discarded due to excessive hemolysis or icterus. Sixteen samples were considered non-neoplastic and sixteen as neoplastic, according to the clinical data of patients and ancillary tests performed previously.

Within the non-neoplastic group samples were classified according to the cause of the effusion, whereas in the neoplastic group samples were classified according to the type of tumor (Table 1).

The CEA (Bioclin[®], BR), CA 72-4 and CYFRA 21-1 (DRG International[®], DE) markers were measured through an ELISA sandwich immunoenzymatic assay (Enzyme Linked Immunosorbent Assay), according to the manufacturer's instructions. For reading of the samples a SpectraMax M5 plate reader and the SoftMax Pro5 software (Molecular Devices[®], US) were used. The results for CEA and CYFRA 21-1 were expressed in nanograms per milliliter (ng/mL) and the results for CA 72-4 were expressed in units per milliliter (U/mL).

Table 1. Distribution of samples of non-neoplastic and neoplastic effusion groups according to the etiology. Samples analyzed in the Laboratory of Veterinary Clinical Analyses at Federal University of Rio Grande do Sul after collection

Non-neoplastic group	n*	Neoplastic group	n*
Rupture of the thoracic duct	1	Mesothelioma	1
Liver failure	4	Carcinoma (2 cholangiocarcinomas and 4 metastatic mammary carcinomas)	6
Nephrotic syndrome	2	Sarcoma (3 hemangiosarcomas, 1 fibrosarcoma, 1 metastatic liposarcoma and 1 metastatic osteosarcoma)	6
Idiopathic pericardial effusion	1	Malignant histiocytosis	1
Heart failure	6	Lymphoma (1 type B and 1 type T)	2
Intestinal parasitosis	2		

*Number of cases.

For comparison of the median levels of the tumor markers between the two groups, the non-parametric Mann-Whitney U test was used. Subsequently, a Receiver Operating Characteristic Curve (ROC curve) analysis was performed for estimation of a cutoff point, area under the curve and levels of accuracy, sensitivity and specificity for each tumor marker with significant differences between groups. For the analyses the statistical program IBM SPSS Statistic 19 was used. In all analyses a significance level of 5% was adopted.

RESULTS

The median values and amplitude of the biochemical tumor markers for the non-neoplastic and neoplastic groups are presented on Table 2. The results for the Mann-Whitney U test for comparison of intervals between the two groups are shown in the same table. Only the CYFRA 21-1 marker showed significant levels ($p=0.001$) in the neoplastic groups when compared to the non-neoplastic.

Table 2. Median and amplitude of the tumor markers for each group and test for comparison of intervals between the two groups

Markers	Group – median (amplitude)		Mann-Whitney		
	Non-neoplastic	Neoplastic	U	z	p
CEA (ng/mL)	0.89 (0.65-6.19)	0.92 (0.61-69.28)	115.50	-0.47	0.637
CYFRA 21-1 (ng/mL)	4.39 (3.31-7.53)	12.14 (4.21-34.68)	37.00	-3.43	0.001
CA72-4 (U/mL)	1.74 (1.15-2.97)	2.23 (1.09-129.70)	99.00	-1.09	0.274

The ROC curve analysis suggested a cutoff point of 6.87ng/mL for the CYFRA 21-1 marker, with a sensitivity level of 70%, specificity of 94% and accuracy of 81%. The area under the curve was

85.5%±0.07 (I.C. 72.5% - 98.6%; $p= 0.001$), as shown in Figure 1. According to these results, one false positive and five false negative cases were observed.

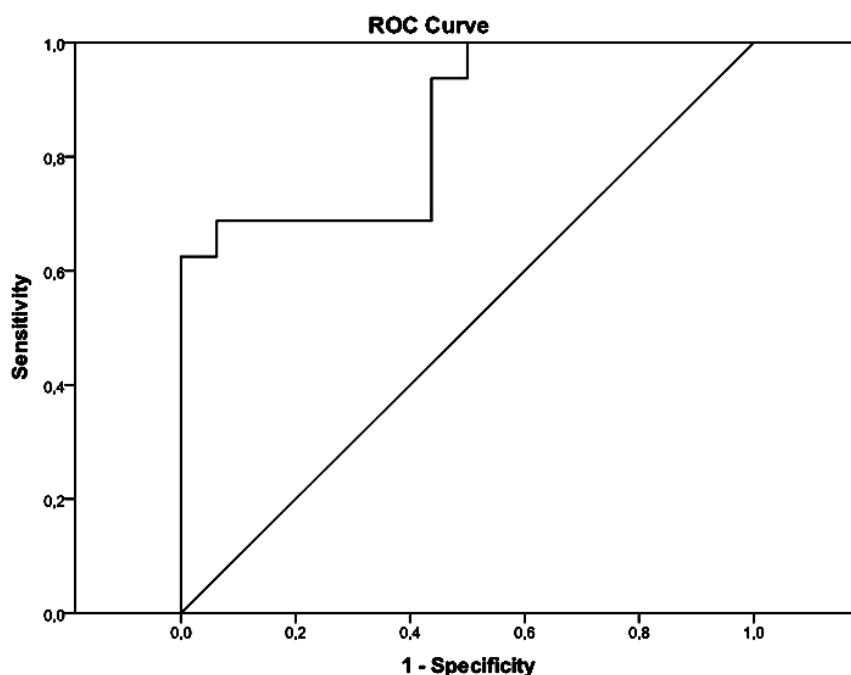


Figure 1. ROC Curve analysis for the CYFRA 21-1 marker. Area under the curve = 85.5% \pm 0.07 (I.C. 72.5% - 98.6%; $p < 0.001$). Sensitivity = 70%, specificity = 94% and accuracy = 81%.

DISCUSSION

The biochemical tumor markers are molecules present in both healthy and sick individuals. However, they are present in higher concentrations in those patients with malignant neoplasms. The increase in the concentration of these markers is associated to a variety of causes, including intense cell exchange, necrosis or increased secretion of certain proteins. Thus, the biochemical tumor markers are widely used as tools for diagnosis and prognosis of cancer patients (Sturgeon, 2002; Trapé *et al.*, 2011; Trapé *et al.*, 2012).

In this study the levels of CEA, CA 72-4 and CYFRA 21-1 in canine neoplastic and non-neoplastic effusions were evaluated. To our knowledge, it is the first time these markers are tested in this species. However, the concentrations of the CEA and CA 72-4 markers did not show significant differences between the non-neoplastic and neoplastic groups. In accordance with Trapé (2011) and colleagues, this event could be explained by the presence of other diseases in the non-neoplastic group, which

may be causing the elevated levels of the markers in these patients.

The CYFRA 21-1 marker showed significantly ($z = -3.43$; $p = 0.001$) higher levels for the neoplastic group in relation to the non-neoplastic group. The cutoff point suggested by the ROC curve analysis presented satisfactory levels of accuracy (81%), sensitivity (70%) and specificity (94%) (Brown and Davis, 2006). However, the marker was more accurate in the diagnosis of positive cases (neoplastic effusions) than negative cases (non-neoplastic effusions). This result suggests that other tests should be considered in the exclusion of neoplasia as a final diagnosis. In our study five cases were false negatives, which corresponded to two lymphomas, two sarcomas and one carcinoma.

Porcel (2004) and colleagues studied the CA 15-3, CA 19-9, CEA and CYFRA 21-1 tumor markers in malignant and benign effusions in humans, concluding that the CYFRA 21-1 marker does not react with tumor cells of non-epithelial origin. However, in their study, samples of four sarcomas and one malignant

histiocytosis showed increased values of this tumor marker, which could be explained by the extensive damage and inflammatory reaction of the mesothelial tissue. Paganuzzi *et al.* (2001) and Suzuki *et al.* (2010) reported that high levels of CYFRA 21-1 are suggestive of mesothelioma, corroborating with our study.

The samples that showed higher levels of the CEA and CA 72-4 markers in the neoplastic group corresponded to the mesothelioma, the malignant histiocytosis and the fibrosarcoma. This indicates that these markers could be specific regarding etiology and extension of the tumor, although the difference in relation to the non-neoplastic effusion group was not significant. In the same way, the number of false negatives (n=5) observed regarding the levels of the CYFRA 21-1 marker suggests that it could be more reactive to certain specific types of illnesses, such as chronic liver and heart diseases. Further studies with more homogeneous samples in relation to tumor type may clarify these issues.

CONCLUSIONS

In our study we concluded that the CA 72-4 and CEA markers do not differentiate between neoplastic and non-neoplastic effusions. The CYFRA 21-1 marker performed well, showing good sensitivity, specificity and accuracy in the diagnosis of malignancies in canine effusions. This study represents an advance in research using biochemical tumor markers in canine effusions. However, further investigations are needed regarding the use of these markers for this species, both in patients with malignancies and in those patients with benign disease.

REFERENCES

- ALATAS, F.; ALATAS, O.; METINTAS, M. *et al.* Diagnostic value of CEA, CA 15-3, CA 19-9, CYFRA 21-1, NSE and TSA assay in pleural effusions. *Lung Cancer*, v.31, p.9-16, 2001.
- BIBBO, M.; LONGATTO FILHO, A. (Ed). *Aspectos Clínicos e Laboratoriais dos Derrames Cavitários – Conduta terapêutica e avaliações diagnósticas e prognósticas*. Rio de Janeiro: Revinter, 2007. 241p.
- BROWN, C.D.; DAVIS, H.T. Receiver operating characteristics curves and related decision measures: A tutorial. *Chem. Int. Lab. Sys.*, v.80, p.24-38, 2006.
- BUCCHERI, G.; FERRIGNO, D. Lung tumor markers of cytokeratin origin: an overview. *Lung Cancer*, v.34, p.65-69, 2001.
- CHAN, D.W.; SCHWARTZ, M.K. Tumor markers: Introduction and general principles. In: DIAMANDIS, E.P.; FRITSCH, H.A.; LILJA, H. *et al.* *Tumor markers: physiology, pathobiology, technology, and clinical applications*. Washington, DC: AACC Press, 2002. p.9-17.
- CLINKENBEARD, K.D. Diagnostic cytology: carcinomas in pleural effusions. *Comp. Cont. Ed. Pract. Vet.*, v.14, p.187-195, 1992.
- COWELL, R.L.; TYLER, R.D.; MEINKOTH, J.H. Lesões cutâneas e subcutâneas. In: COWELL, R.L. *Diagnóstico citológico e hematologia de cães e gatos*. São Paulo: Medvet, 2009. p.78-111.
- DEJSOMRITRUTAI, W.; SENAWONG, S.; PRONKIAMON, B. Diagnostic utility of CYFRA 21-1 in malignant pleural effusion. *Resp.*, v.6, p.213-216, 2001.
- EDWARDS, N.J. The diagnostic value of pericardial fluid pH determination. *J. Am. Anim. Hosp. Assoc.*, v.32, p.63-67, 1996.
- FENTON, N.K.; RICHARDSON, J.D. Diagnosis and management of malignant pleural effusions. *Am. J. Surg.*, v.170, p.69-74, 1995.
- FERRER, J.S. Marcadores tumorales em líquido pleural. *Arch. Bronc.*, v.36, p.295-297, 2000.
- FERRER, J.; VILLARINO, M.A.; ENCABO, G. *et al.* Diagnostic utility of CYFRA 21-1, carcinoembryonic antigen, CA 125, neuron specific enolase, and squamous cell antigen level determinations in the serum and pleural fluid of patients with pleural effusions. *Cancer*, v.86, p.1488-1495, 1999.
- GROSS, J.L. *Marcadores tumorais séricos em pacientes com carcinoma de pulmão não pequenas células*. 1999. 103f. Dissertação (Mestrado em Ciências Médicas). Faculdade de Medicina, Universidade de São Paulo, São Paulo.

- HAHN, K.A.; RICHARDSON, R.C. Detection of serum Alpha-Fetoprotein in dogs with naturally occurring malignant neoplasia. *Vet. Clin. Path.*, v.24, p.18-24, 1995.
- KRUTH, A.S. Abdominal Distention, Ascitis, And Peritonitis. In: ETTINGER, S.J.; FELDMAN, E.C., *Textbook of Veterinary Internal Medicine*. Philadelphia: WB Saunders Company, 2004. p.150-153.
- KUMAR, P.; PAWAIYA, R.V.S. Advances in cancer diagnostics. *Braz. J. Vet. Pathol.*, v.3, p.142-153, 2010.
- LECHOWSKI, R.; JAGIELSKI, D.; HOFFMANN-JAGIELSKA, M. *et al.* Alpha-fetoprotein in canine multicentric lymphoma. *Vet. Res. Com.*, v.26, p.285-296, 2002.
- LIGHT, R.W. Pleural effusion related to metastatic malignancies. In: LIGHT, R.W. (Ed). *Pleural diseases*. Philadelphia: Lippincott, 2001. p.108-134.
- LOWSETH, L.A.; GILLET, N.A.; CHANG, I.Y. *et al.* Detection of alpha-fetoproteina in dog with hepatic tumors. *J. Am. Vet. Med. Assoc.*, v.199, p.735-741, 1991.
- MIÉDOUGÉ, M.; ROUZAUD, P.; SALAMA, G. *et al.* Evaluation of seven tumor markers in pleural fluid for the diagnosis of malignant effusions. *Brit. J. Cancer*, v.81, p.1059-1065, 1999.
- O'BRIEN, P.J.; LUMSDEN, J.H. The cytologic examination of body cavity fluids. *Sem. Vet. Med. Surg.*, v.3, p.140-156, 1988.
- PAGANUZZI, M.; ONETTO, M.; MORRONI, P. *et al.* Diagnostic value of CYFRA 21-1 tumor marker and CEA in pleural effusion due to mesothelioma. *Chest.*, v.119, p.1138-1142, 2001.
- PORCEL, J.M.; VIVES, M.; ESQUERDA, A. *et al.* Use of a panel of tumor markers (carcinoembryonic antigen, câncer antigen 125, carbohidrate antigen 15-3, and cytokeratin 19 fragments) in pleural fluid for the differential diagnosis of benign and malignant effusions. *Chest.*, v.126, p.1757-1763, 2004.
- RASKIN, R.E.; MEYER, D.J. *Atlas of Canine and Feline Cytology*. Philadelphia: WB Saunders Company, 2001. 430p.
- ROMERO, S.; FERNÁNDEZ, C.; ARRIERO, J.M. *et al.* CEA, CA 15-3 and CYFRA 21-1 in serum and pleural fluid of patients with pleural effusion. *Eur. Res. J.*, v.9, p.17-23, 1996.
- STURGEON, C. Practice guidelines for tumor marker use in the clinic. *Clin. Chem.*, v.48, p.1151-1159, 2002.
- SUZUKI, H.; HIRASHIMA, T.; KOBAYASHI, M. *et al.* Cytokeratin 19 Fragment/ Carcinoembryonic Antigen ratio in pleural effusion is a useful marker for detecting malignant pleural mesothelioma. *Anticancer Res*, v.30, p.4343-4346, 2010.
- TRAPÉ, J.; FILELLA, X.; ALSINA-DONADEU, M. *et al.* Increased plasma concentrations of tumor markers in the absence of neoplasia. *Clin. Chem. Lab. Med.*, v.49, p.1605-1620, 2011.
- TRAPÉ, J.; MOLINA, R.; SANT, F. Clinical evaluation of the simultaneous determination of tumor markers in fluid and serum and ratio in the differential diagnosis of serous effusions. *Tumor Biol.*, v.25, p.276-281, 2004.
- TRAPÉ, J.; MOLINA, R.; SANT, F. *et al.* Diagnostic accuracy of tumour markers in serous effusions: a validation study. *Tumor Biol.*, v.33, p.1661-1668, 2012.
- VILLENA, V.; LOPEZ-ENCUENTRA, A.; ECHAVE-SUSTAETA, J. *et al.* Diagnostic value of CA 72-4, carcinoembryonic antigen, CA 15-3, and CA 19-9 assay in pleural fluid: A study of 207 patients. *Cancer*, v.78, p.736-740, 1996.
- VILLENA, V.; LOPEZ-ENCUENTRA, A.; ECHAVE-SUSTAETA, J. *et al.* Diagnostic value of CA 549 in pleural fluid. Comparison with CEA, CA 15-3 and CA 72-4. *Lung Cancer*, v.40, p.289-294, 2003.