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Arq. Bras. Med. Vet. Zootec., v.76, n.5, e13231, 2024

http://dx.doi.org/10.1590/1678-4162-13231
Original Article - Veterinary Medicine

Clinical value of alpha-fetoprotein in the detection of mammary carcinoma in female dogs

[*Valor clínico da alfafetoproteína na detecção de carcinoma mamário em cadelas*]

I.L.S. *Senhorello*[1,2], E.M. *Terra*[3], F.A.R. *Sueiro*[4], R.S. *Pereira*[4], B.F. *Firmo*[2], C. *Martinelli*[5], M. *Tinucci-Costa*[2]

[1]Faculdade de Medicina Veterinaria de Araçatuba, Universidade Estadual Paulista, Araçatuba, SP, Brasil
[2]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brasil
[3]Veterinarian, Altinópolis, SP, Brasil
[4]Veterinarian, Campinas, SP, Brasil
[5]Universidade Vila Velha, UVV, Vila Velha, ES, Brasil

ABSTRACT

This study aimed to assess the behavior of Alpha-Fetoprotein (AFP) in healthy female dogs, those with non-metastatic mammary carcinomas, and those with metastatic mammary carcinomas. Additionally, it aimed to evaluate serum levels concerning the clinical-pathological variables of diseased dogs. To achieve this, sera from 35 female dogs were utilized, divided into three groups: G1 (n=10), control group (dogs free of neoplasms); G2 (n=20), dogs with mammary carcinoma without lymph node metastasis; and G3 (n=5), dogs with mammary carcinoma with lymph node metastasis. AFP was measured through ELISA, and the results were assessed using the Tukey test with a significance level of 5% in terms of the marker's presence in the patient's serum, its relationship with the neoplasm's biological behavior, and the clinical-pathological alterations encountered. Additionally, sensitivity and specificity values were obtained to establish the clinical value of AFP as a serological marker. The results revealed that AFP values ($p<0.001$) were significantly higher in dogs with mammary carcinoma compared to healthy dogs. Furthermore, there was a significant increase in AFP in non-spayed animals ($p=0.0307$). The marker demonstrated a sensitivity of 92% and specificity of 90% in distinguishing diseased animals from healthy ones. No relationship was found between the variables of tumor size, lymph node metastasis, histological grade, necrosis, ulceration, and inflammation with AFP ($p<0.05$). The findings indicated that AFP is elevated in female dogs with mammary tumors and could be a promising marker for monitoring dogs with mammary neoplasms. Future studies that include patient follow-up will be necessary.

Keywords: prognosis, mammary tumor, biomarkers, metastasis

RESUMO

Esse estudo teve como objetivo avaliar o comportamento da alfafetoproteína (AFP) em cadelas saudáveis, com carcinomas mamários não metastáticos e naquelas com carcinomas mamários metastáticos. Adicionalmente, objetivou-se avaliar os níveis séricos quanto às variáveis clínico-patológicas dos cães doentes. Para tanto, foram utilizados soros de 35 cadelas, divididas em três grupos: G1 (n=10), grupo controle (cadelas livres de neoplasias); G2 (n=20), cadelas com carcinoma mamário sem metástase linfonodal; e G3 (n=5), cadelas com carcinoma mamário com metástase linfonodal. A AFP foi medida por ELISA, e os resultados avaliados pelo teste de Tukey, com nível de significância de 5%, quanto à presença do marcador no soro dos pacientes, à sua relação com o comportamento biológico da neoplasia e às alterações clínico-patológicas encontradas. Além disso, foram obtidos valores de sensibilidade e especificidade para estabelecer o valor clínico da AFP como marcador sorológico. Os resultados revelaram que os valores de AFP (P<0,001) foram significativamente maiores em cadelas com carcinoma mamário, em comparação com cadelas saudáveis. Ademais, houve aumento significativo de AFP em animais não esterilizados (P=0,0307). O marcador demonstrou sensibilidade de 92% e especificidade de 90% para distinguir animais doentes de saudáveis. Não foi encontrada relação

Corresponding author: salardani@hotmail.com
Submitted: February 20, 2024. Accepted: April 16, 2024.

entre as variáveis tamanho do tumor, metástase linfonodal, grau histológico, necrose, ulceração e inflamação com AFP (P<0,05). Os resultados indicaram que a AFP está elevada em cadelas com tumores mamários e pode ser um marcador promissor para o monitoramento de cadelas com neoplasias mamárias. Estudos futuros que incluam o acompanhamento dos pacientes serão necessários.

Palavras-chave: prognóstico, tumor mamário, biomarcadores, metástase

INTRODUCTION

Mammary tumors are highly prevalent in female dogs and exhibit a biological behavior similar to that in women, making the dog a model for comparative studies. However, unlike in women, monitoring methods for the disease and more concrete prognosis estimation are lacking in dogs. In women, the use of serological markers is well-established, providing a useful method for patient monitoring from the moment of diagnosis (Lindblad-toh *et al*., 2005; Wang *et al.*, 2014).

The mammary gland is the most frequent site of tumors in female dogs, especially in adult and intact ones. The incidence is three times higher than in women, and about 50% of these cases are malignant (Uva *et al*., 2009; Rasotto *et al*., 2012).

Female dogs with malignant mammary neoplasms present significantly shorter survival compared to those with benign neoplasms. A common cause of mortality due to this disease, both in women and female dogs, is the occurrence of recurrences and metastases from the primary tumor, primarily to regional lymph nodes, lungs, liver, and spleen (De Nardi *et al*., 2016; Klopfleisch and Gruber, 2009). According to Sorenmo (2003), the two-year survival rate for female dogs with malignant neoplasms is 25% to 40%. However, this survival rate can be influenced by various factors and can vary significantly depending on the type and histological grade of the tumor, the presence of regional or distant metastases, the disease stage, and the treatment used.

The search for initiatives and methods that aid in diagnosis, monitoring, and prognosis determination for patients is ongoing. Prognostic factors can be established based on clinical, pathological, and biological characteristics or a combination of these specific to individuals and their tumors. These factors allow for predicting the clinical course and survival of patients after initial surgery (Cavalcanti and Cassali, 2006; Cassali *et al*., 2020).

Although various prognostic markers are established in human medicine, the search for possible markers in veterinary oncology is ongoing, with most obtained through immunohistochemistry. In this way, these markers, when expressed in the tumor, are assessed at a single time point, typically when the primary tumor is surgically removed (De Nardi *et al*., 2007; Cassali *et al*., 2020).

In humans, both for breast cancer and other neoplasms, the use of serological markers was introduced to aid in patient monitoring from diagnosis to the end of treatment. They are useful for the early detection of recurrences and contribute to reducing the physical and emotional strain associated with the exams used for patient monitoring. Furthermore, these markers are known to be related to patient prognosis (Harris *et al*., 2007).

Serological markers are proteins or enzymes produced by tumor cells in response to tumorigenesis and are present in the patient's serum (Uehara *et al*., 2008). These enzymes or proteins vary depending on the tumor type and affected organ (Harris *et al*., 2007), and increased concentrations can indicate tumor origin, recurrence, or growth (Capelozzi, 2001).

One serological marker associated with breast cancer in women is alpha-fetoprotein (AFP). According to Sarcione and Biddle (1987), AFP is increased in the serum of women with primary mammary carcinoma, and its serial evaluation can provide important information regarding patient diagnosis and prognosis. AFP is a significant fetal serum protein synthesized in the liver, yolk sac, and fetal intestines, with functions in plasma transport and maintenance of oncotic pressure decreasing in the first year of life (Bennett *et al*., 1997). In dogs, elevated serum AFP levels have been linked to the

presence of hepatic carcinoma and cholangiocarcinoma (Yamada *et al*., 1999).

Despite being widely used in human medicine, the use of serological markers for monitoring neoplasms and determining prognosis is not yet a reality in veterinary medicine, although they are being tested with some promising results (Manuali *et al*., 2012; Senhorello *et al*., 2020; Colombe *et al*., 2022). Female dogs with mammary tumors undergo surgery after undergoing periodic staging exams and disease monitoring (Cassali *et al*., 2020). The potential use of less invasive markers that can early indicate tumor recurrence or metastatic focus is becoming increasingly useful and necessary.

Therefore, this study aimed to assess the behavior of AFP in female dogs with metastatic and non-metastatic mammary carcinomas before surgical treatment and to correlate its concentrations with clinical-pathological variables such as tumor size, histological grade, presence of necrosis, ulceration, inflammation, and the influence of spaying. The intention is to potentially use these markers for patient monitoring and prognosis in the future.

METHODS AND MATERIALS

The current investigation underwent scrutiny by the Animal Experimentation Ethics Committee of the Faculty of Agricultural and Veterinary Sciences at UNESP-Jaboticabal (protocol n. 10801/15). Participation of project contributors was contingent upon the provision of informed consent, with each contributor duly signing an informed consent form.

Thirty-five female dogs, devoid of breed or age preferences, were enlisted for this research. These dogs were procured from the Veterinary Oncology and Obstetrics Services at the Veterinary Hospital of UNESP, Jaboticabal campus, SP, Brazil. Upon initial diagnosis, 5.0 mL of blood was procured through jugular venipuncture, followed by centrifugation to extract serum. Subsequently, the sera were individually aliquoted and preserved at -20 °C for subsequent quantification of serological markers.

The control group consisted of blood samples from ten healthy dogs aged between 1 and 12 years (4 ± 3.2), without a history of neoplasia, undergoing elective castration or immunization at the Veterinary Hospital of UNESP, Jaboticabal, SP, Brazil. These animals underwent routine laboratory tests, three-projection chest radiographs, and abdominal ultrasound to confirm their health status. Blood collection followed the same protocol.

The collected biological material was categorized into three experimental groups based on the TNM clinical staging of the World Health Organization (WHO) guidelines (Owen, 1980):

- Group 1 (G1): Control group encompassing 10 serum aliquots from healthy female dogs without a neoplasm history.
- Group 2 (G2): Comprising 20 serum aliquots from female dogs with mammary carcinomas without metastasis, according to clinical staging.
- Group 3 (G3): Composed of five serum aliquots from female dogs with mammary carcinomas exhibiting regional lymph node metastasis, as determined through histological evaluation.

Patient selection occurred post-clinical staging, involving a detailed physical examination at the time of diagnosis, conforming to semiological precepts (mammary gland palpation, nodule measurement, evaluation of patient general condition), routine laboratory tests, three-projection thoracic radiographs, and abdominal ultrasonography for metastasis screening. TNM clinical staging adhered to the guidelines of the World Health Organization (WHO) (Owen, 1980).

Total or unilateral mastectomy was performed with regional lymph node removal (inguinal and/or axillary) for histopathological evaluation and tumor staging in accordance with the specific indication for each case (Cassali *et al*., 2020).

Clinicopathological variables, including tumor size and presence of ulceration (macroscopic evaluation), histological grade, necrosis, inflammation (histopathological evaluation), and previous ovariohysterectomy, were assessed. Inflammation was determined by the presence of moderate to intense peritumoral and intratumoral lymphoplasmacytic infiltrate. Tumor size was measured as the largest tumor diameter in centimeters (cm) using a digital caliper

(Yamagami *et al*., 1996). In cases where bitches had multiple breast lumps, the largest lump was considered for tumor size evaluation.

To evaluate the correlation between AFP concentrations and tumor size, serum samples from G2 na G3 bitches were categorized into three groups (n = 25):

- T1: Serum samples from bitches with tumors smaller than 3.0 cm (n = 14).
- T2: Serum samples from bitches with tumors between 3.0 and 5.0 cm (n = 6).
- T3: Serum samples from bitches with tumors larger than 5.0 cm (n = 5).

The study included bitches with metastasis detectable only in the regional lymph node, excluding those with images suggestive of distant metastasis at the time of diagnosis. Distant metastasis was defined as masses in the thoracic or abdominal cavity detected by radiographic and/or ultrasonographic imaging. This exclusion criterion allowed for an assessment of the tumor's influence on AFP dosage since, predominantly, only animals without distant metastasis undergo mastectomy, although a few bitches with distant metastasis received clinical care at the University during the project.

The influence of hepatic alterations was also considered as an exclusion criterion. Patients showing any abnormalities in serum biochemical tests reflecting signs of hepatic injury and/or functional changes were excluded. The tests included in this evaluation were alanine aminotransferase, aspartate transaminase, alkaline phosphatase, gamma-glutamyl transferase, albumin, cholesterol, and bilirubin levels. Furthermore, any abnormalities in ultrasonographic images suggestive of acute or chronic liver diseases also met the exclusion criteria. This assessment was performed on patients from both experimental groups. Additional exclusion criteria encompassed the presence of other neoplasms and concomitant diseases unrelated to mammary neoplasia that could potentially interfere with marker concentration.

Immediately post-surgical removal of the mammary chain and draining lymph node, breast, and lymph node samples were fixed in 10% buffered formalin for 24 to 48 hours and subsequently stored in 70% alcohol until processing. Following this, samples were processed, embedded in paraffin, and 5 µ-thick sections were cut using a microtome, followed by Hematoxylin & Eosin staining. Light microscopy was employed to assess cell differentiation degree, pleomorphism, anaplasia, metastasis in blood and lymph vessels, invasion of adjacent tissue, and lymph node metastasis (Cassali *et al*., 2020). Regarding histological grade, groups were classified into grades I, II, and III (Elston and Ellis, 1998.

The serological marker AFP was determined using a human ELISA kit (AccuBind ELISA Kits, MONOBIND INC, catalog 1925-300). For the assays, 300µL of blood serum from each group was tested using the Elisys Uno Human® reading equipment, following the manufacturer's recommended technique. Studies using antibodies for human AFP have demonstrated a good affinity for detecting canine AFP, validating the use of the interspecies kit (Madsen and Rikkers, 1984 Madsen *et al*., 1980 Lowseth *et al*., 1991.

Variables underwent analysis of variance with a completely randomized design and a significance level of 5%. Variables with high coefficients of variation were transformed using the natural logarithm of the response measure + 1 before analysis. Multiple mean comparisons were conducted using the Tukey test (significance level = 0.05). The diagnostic value of the serum tumor marker was evaluated through the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, and the cut-off value based on the control group was established. The General Linear Models (GLM) procedure of the SAS computational program (SAS 9.1, SAS Institute, Cary, NC, USA) was employed for these analyses.

RESULTS

The histological type most frequently diagnosed was mixed tumor carcinoma (Cassali *et al*., 2020), accounting for 60% (15/25) of cases, followed by tubular carcinoma at 16% (4/25) (Table 1). Regarding the breed frequency, mixed-breed animals (13/25; 52%) were the majority, and the age ranged from 5 to 15 years (mean of 10 ± 2.42).

Table 1. Histopathological classification of the mammary tumors in the dogs of this study (n=25)

Diagnosis	Frequency	%
Mixed tumor carcinoma	15	60
Tubular carcinoma	4	16
Papillary carcinoma	3	12
Solid carcinoma	2	8
Micropapillary carcinoma	1	4
Total	25	100

The mean concentrations of AFP were significantly higher ($p<0.001$) in diseased animals (1.57±1.15ng/mL) compared to healthy animals (0.57 ± 0.21ng/mL) (Figure 01). Concerning the experimental groups, AFP concentrations differed between the means of groups G1 and G2 ($p<0.001$) and G1 and G3 ($p<0.001$), where dogs with metastatic or non-metastatic mammary carcinoma had higher AFP values than the control group. There was no significant difference between groups G2 and G3 ($p>0.05$) (Fig. 1 and Table 2).

Table 2. Mean and standard deviation of AFP serum levels in healthy female dogs (G1), those with mammary carcinomas without metastasis (G2), and those with mammary carcinomas with metastasis (G3)

Groups (n)	AFP (T) (ng/ml)
G1 (10)	0.57 (±0.21) a
G2 (20)	1.60 (±1.15) b
G3 (5)	1.42 (±1.14) b

AFP: Alpha-fetoprotein; Different letters indicate statistical difference among the means ($p<0.05$); Same letters indicate no difference among the means ($p>0.05$). *T- Logarithmic transformation was applied to AFP values.

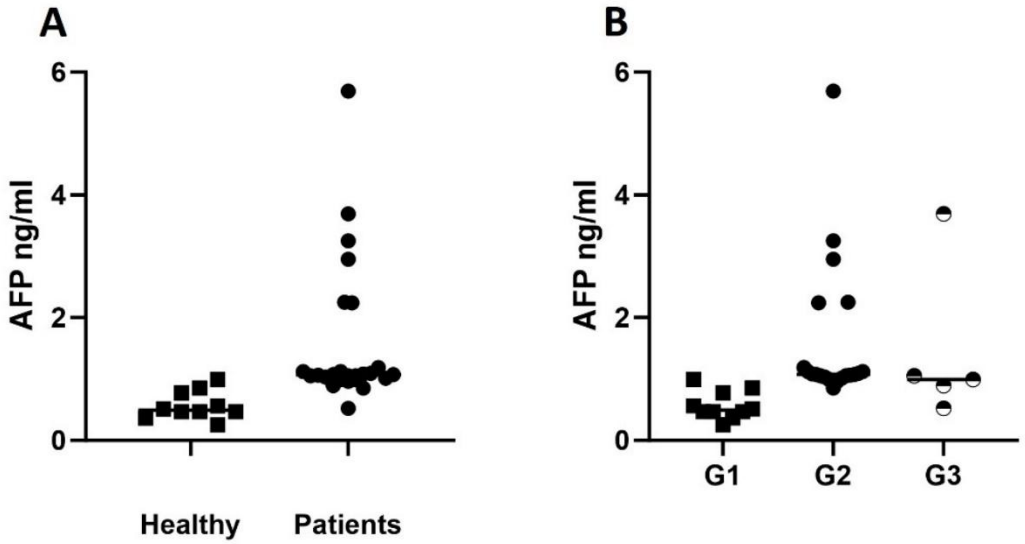

Figure 1. Graphical representation of serum AFP levels in healthy and diseased dogs (A) and segmented into groups G1, G2, and G3 (B).

Figure 2 shows the area under the ROC curve concerning healthy animals. The best cutoff value was estimated to maximize the sum of sensitivity and specificity in diagnosing an animal with a mammary gland tumor. The serum AFP values established from the ROC curve were 0.87ng/mL, with a sensitivity of 92% (CI 75.03% to 98.58%) and specificity of 90% (CI 59.58% to 99.49%).

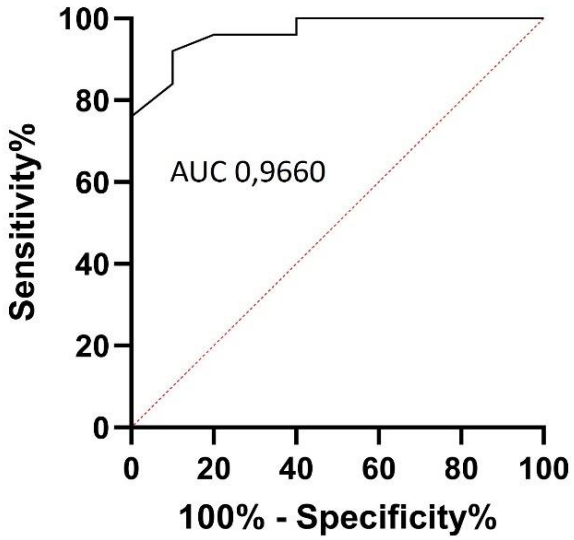

Figure 2. Receiver Operating Characteristic (ROC) curve for AFP (ng/mL) and Area Under the ROC Curve (AUC).

No differences were observed in AFP levels concerning the clinical-pathological variables used in this study, including tumor size, histological grade, ulceration, necrosis, and inflammation (p>0,05). Regarding neutering, a significant difference in AFP concentrations was observed between spayed and non-spayed animals, with spayed females having lower AFP values (p=0.0307). (Table 3).

Table 3. Relationship between serum AFP concentrations and analyzed clinical-pathological variables (mean and standard deviation)

Variables		AFP (ng/ml)	P-value
Tumor Size	T1 (n=14)	1.32 (±0.75)a	0.1614
	T2 (n=6)	2.23 (±1.79)a	
	T3 (n=5)	1.13 (±0.25)a	
Histological Grade	I (n=8)	1.06 (±0.03)a	0.42
	II (n=8)	1.83 (±1.70)a	
	III (n=9)	1.55 (±0.83)a	
Ulceration	No (n= 20)	1.59 (±1.15)a	0.3411
	Yes (n= 5)	1.02 (±0.14)a	
Necrosis	No (n= 19)	1.64 (±1.21)a	0.2637
	Yes (n= 6)	1.06 (±0.07)a	
Inflammation	No (n=10)	1.38 (±0.68)a	0.65
	Yes (n=15)	1.58 (±1.29)a	
Neutering	No (n=20)	2.42 (±1.93)a	0.0307*
	Yes (n=5)	1.27 (±0.64)b	

AFP: Alpha-fetoprotein; Different letters indicate statistical difference among the means (p<0.05); Same letters indicate no difference among the means (p>0.05).

DISCUSSION

The present study aimed to investigate the behavior of Alpha-Fetoprotein (AFP) in female dogs with mammary tumors, including both non-metastatic and metastatic cases, and to assess its association with various clinical-pathological variables. Our findings contribute to a better understanding of the potential role of AFP as a serological marker in canine mammary neoplasms.

Serum levels of AFP were detectable in all animals in this study using the ELISA technique and a commercial human kit. It has been demonstrated that assays using a monospecific rabbit anti-serum for human AFP have a strong affinity for canine AFP, both by radioimmunoassay techniques and gel diffusion (Madsen & Rikkers, 1984 Madsen *et al*., 1980). Another study using commercial human kits, one for radioimmunoassay and the other for enzymatic test, was able to detect canine AFP (Lowseth *et al*., 1991.

The AFP assays were useful in differentiating healthy and diseased dogs, given the high sensitivity and specificity when comparing serum levels in the two groups. However, there was no difference in serum levels between the groups with lymph node metastasis (G3) and those without metastasis (G2). The first study involving AFP in mammary neoplasms in women was conducted by Sarcione and Biddle (1987), showing similar results to those found in our study. The authors concluded that AFP was elevated in the serum of women with primary breast tumors, suggesting that serial measurements could provide important information regarding the diagnosis and prognosis of this type of neoplasm.

On the other hand, in a study involving a panel of serological markers, including AFP, in women with breast neoplasms, researchers observed no difference in AFP values between women with breast neoplasms and healthy women, concluding that this marker would not be useful for monitoring breast cancer progression in women (Opstal-Van Winden *et al*., 2012). However, in hepatic, gastric, and colorectal carcinomas in humans, AFP production by the tumor directly influences the more aggressive behavior of neoplasms and, consequently, patient prognosis (Ren *et al*., 2019; Głowska-Ciemny *et al*., 2023; Mao *et al*., 2023). In our study, it was not possible to observe an increase in AFP in relation to clinicopathological variables such as histological grade, tumor size, necrosis, and inflammation. However, a limitation of this assertion is the relatively small number of evaluated patients, requiring a larger-scale evaluation to confirm these data.

In female dogs with mammary neoplasms, there are no previous studies evaluating AFP as a possible serological marker. However, studies in dogs with hepatocellular carcinoma, cholangiocarcinoma, and lymphoma have shown an increase in this marker in affected patients (Lowseth *et al*., 1991; Hahn and Richardson, 1995; Yamada *et al*., 1999). Furthermore, Kitao *et al*. (2006) observed that dogs with hepatic neoplasms with high serum levels of AFP had protein expression in the tumor. Additionally, Marchesi *et al*. (2007) evaluated AFP in the plasma and serum of healthy dogs, non-neoplastic diseases, and various types of neoplasms (mast cell tumor, lymphoma, carcinoma, sarcoma) and found an increase in the marker in dogs with neoplastic diseases compared to the other groups.

Patients in our study were subjected to a rigorous selection criterion, as hepatic diseases could drastically affect the marker values. This allowed us to mitigate interferences from concurrent diseases in this assessment. It is worth noting that one animal from group G2 had an AFP value of 5.69 ng/ml, which significantly differed from the values of the other animals in the group. We sought additional information about this patient, and interestingly, we observed that the tumor presented important histopathological characteristics of inflammation and measured approximately 4.5 cm in its largest axis. Furthermore, the dog had been spayed years before developing mammary neoplasia and strictly met the selection criteria, so she was included in the statistical analyses.

Furthermore, the reproductive status of the female dogs was related to AFP values. Female dogs with mammary carcinoma and spayed had significantly lower AFP values than intact ones. AFP is a naturally occurring antiestrogenic protein, and its concentrations correlate with the estrous and reproductive cycle in women (Bennet

et al., 1997). Therefore, our results suggest that the same may occur in female dogs. In rats, Uriel *et al*. (1976) observed a connection between AFP and estrogen and that this important fetal protein assists in the transport of estrogen into uterine cells in these animals. Years later, Pool *et al*. (1978) observed an influence on the estrous cycle of rats. The authors suggested that there is a connection between AFP and estrogen, with consequent inhibition of its action.

In addition, besides serum AFP levels being hormonally influenced in women, elevated values during pregnancy correlate with a lower incidence of breast tumors, probably due to the antiestrogenic effect (Richardson *et al*., 1998). Several pieces of evidence demonstrate the anticancer effect of AFP in humans (Villacampa *et al*., 1984; Bennett *et al*., 1998, 2023; Głowska-Ciemny *et al*., 2023). Future research regarding this variable in female dogs seems rational, given the similarity found. Furthermore, studies correlating AFP levels with hormonal status in the tumor and molecular classification, as well as survival time, are necessary to advance comparative oncology and understand the role of AFP in female dogs with mammary tumors. Considering that a peptide that mimics the antiestrogenic effect of AFP was recently developed and tested in dogs, showing good pharmacokinetic parameters and tolerance (Bennett *et al*., 2023).

Other serological markers such as carcinoembryonic antigen (CEA) and cancer antigen 15.3 (CA 15.3) were evaluated in female dogs with mammary tumors, and similar to what occurs in women, these markers are also elevated in sick dogs (Campos *et al*., 2012; Senhorello *et al.*, 2020; Jain *et al*., 2021; Yang *et al*., 2023). Another relevant finding is that CEA decreases after tumor removal and is more elevated in dogs with lymph node metastasis, making it a promising serological marker for patient monitoring (Senhorello *et al*., 2020). In this study, it was not possible to evaluate AFP simultaneously with other well-known promising markers. However, the inclusion of AFP in the assessment panel for female dogs with mammary tumors is suggested for future research.

It becomes necessary to monitor a larger number of female dogs with mammary carcinomas, where the clinical and laboratory follow-up of these animals can also be included in the study, with repeated marker measurements. If the results are consistent, introducing these assays into the clinical routine of veterinary oncology in the future will be beneficial.

Our results demonstrate that this marker has potential utility in female dogs with mammary neoplasms, as it showed high sensitivity and specificity in distinguishing between female dogs with or without mammary tumors. However, further investigation is needed to confirm its potential as a serological marker in female dogs with mammary neoplasms and to understand the hormonal influence on serum levels.

FUNDING

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), process 2015/15015-9 and 2016/00128-5.

ACKNOWLEDGMENTS

The authors thank theCoordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001.

REFERENCES

BENNETT. J.A.; HOHENHAUS, A.; ANDERSEN, T.T. Proof-of-Concept study of an alpha-fetoprotein-derived peptide for the management of canine mammary cancer. *Animals*, 2023 v.13, p.403, 2023.

BENNETT, J.A.; SEMENIUK, D.J.; JACOBSON, H.I.; MURGITA, R.A. Similarity between natural and recombinant human alpha-fetoprotein as inhibitors of estrogen-dependent breast cancer growth. *Breast Cancer Res. Treat*., v.45, p.169-179, 1997.

BENNETT, J.A.; ZHU, S.; PAGANO-MIRARCHI, A.; KELLOM, T.A.; JACOBSON, H.I. Alpha-fetoprotein derived from a human hepatoma prevents growth of estrogen-dependent human breast cancer xenografts. *Clin. Cancer Res. Official J. Am. Assoc. Cancer Res*., v.4, p.2877-2884, 1998.

CAMPOS, L.C.; LAVALLE, G.E.; ESTRELA-LIMA, A. *et al*. CA 15.3, CEA and LDH in dogs with malignant mammary tumors. *J. Vet. Intern. Med*., v.26, p.1383-1388, 2012.

CAPELOZZI, V.L. Understanding the role of biological markers in lung cancer. *Journal of Pulmonology*, v. 27, p. 321-328, 2001.

CASSALI, G.D.; JARK, P.C.; GAMBA, C. *et al*. Consensus regarding the diagnosis, prognosis and treatment of canine and feline mammary tumors-2019. *Braz. J. Vet. Pathol*., v.13, p.555-574, 2020.

CAVALCANTI, M.F.; CASSALI, G.D. Prognostic factors in the clinical and histopathological diagnosis of mammary tumors in female dogs - review. *Rev. Clín. Vet*., v.61, p.56-63, 2006.

COLOMBE, P.; BÉGUIN, J.; BENCHEKROUN, G.; LE ROUX, D. Blood biomarkers for canine cancer, from human to veterinary oncology. *Vet. Comp. Oncol*., v.20, p.767-777, 2022.

DE NARDI, A.B.; RODASKI, S.; ROCHA, N.S.; FERNANDES, S.C. Breast neoplasms. In: DALECK, C.R.; DE NARDI, A.B. (Eds.). *Oncology in dogs and cats*. São Paulo: Roca, 2016. p.372-383

DE NARDI, A.; DALECK, C.; LAUFERAMORIM, R. *et al*. Correlation of Cyclooxygenase-2 with the prognosis of mammary carcinomas in female dogs. *Acta Sci. Vet*., v.35, p.619-627, 2007.

ELSTON, C.W.; ELLIS, I. O. Assessment of histological grade. In: ELSTON, C.W.; ELLIS, I.O. (Eds.). *Systemic pathology*. The breast. London: Churchill Livingstone, 1998. p.365-384.

GŁOWSKA-CIEMNY J, SZYMAŃSKI M, KUSZERSKA A. *et al*. The Role of Alpha-Fetoprotein (AFP) in contemporary oncology: the path from a diagnostic biomarker to an anticancer drug. *Int. J. Mol. Sci*., v.24, p.2539, 2023.

HAHN, K.A.; RICHARDSON, R.C. Detection of serum alpha-fetoprotein in dogs with naturally occurring malignant neoplasia. *Vet. Clin. Pathol*., v.24, p.18-21, 1995.

HARRIS, L.; FRITSCHE, H.; MENNEL, R. *et al*. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J. Clin. Oncol*., v.25, p.5287-5312, 2007.

JAIN, M.; INGOLE, S.D.; DESHMUKH, R.S. *et al*. CEA, CA 15-3, and miRNA expression as potential biomarkers in canine mammary tumors. *Chromosome Res*., v.29, p.175-188, 2021.

KITAO, S.; YAMADA, T.; ISHIKAWA, T. *et al*. Alpha-fetoprotein in serum and tumor tissues in dogs with hepatocellular carcinoma. *J. Vet. Diagn. Invest*., v.18, p.291-295, 2006.

KLOPFLEISCH, R.; GRUBER, A.D. Differential expression of cell cycle regulators p21, p27 and p53 in metastasizing canine mammary adenocarcinomas versus normal mammary glands. *Res. Vet. Sci*., v.87, p.91-96, 2009.

LINDBLAD-TOH, K.; WADE, C.M.; MIKKELSEN, T.S. *et al*. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*, v.438, p.803-819, 2005.

LOWSETH, L.A.; GILLETT, N.A.; CHANG, I.Y.; MUGGENBURG, B.A.; BOECKER, B.B. Detection of serum alpha-fetoprotein in dogs with hepatic tumors. *J. Am. Vet. Med. Assoc*., v.199, p.735-741, 1991.

MADSEN, A.C.; RIKKERS, L.F. α-Fetoprotein secretion by injured and regenerating hepatocytes in the dog. *J. Surg. Res*., v.37, p.402-408, 1984.

MADSEN, A.C.; RIKKERS, L.F.; MOODY, F.G.; WU, J.T. α-Fetoprotein as a marker for hepatic regeneration in the dog. *J. Surg. Res*., v.28, p.71-76, 1980.

MANUALI, E.; DE GIUSEPPE, A.; FELIZIANI, F. *et al*. CA 15-3 cell lines and tissue expression in canine mammary cancer and the correlation between serum levels and tumour histological grade. *BMC Vet. Res*., v.8, p.86, 2012.

MAO, X.; WANG, J.; LUO, F. α-Fetoprotein contributes to the malignant biological properties of AFP-producing gastric cancer. *Open Life Sci*., v.18, p.20220476, 2023.

MARCHESI, M.C.; CONTI, M.B.; PIERAMATI, C. *et al.* Assessment and behavior of alphafetoprotein (AFP), antigen cancer 15/3 (CA 15/3), carcinembryonal antigen (CEA) in clinical oncology of the dog: preliminary study. *Vet. Res. Commun*.,v.31 Suppl.1, p.301-304, 2007.

MARCHESI, M.C.; MANUALI, E.; PACIFICO, E. *et al*. Cancer antigen 15/3: possible diagnostic use in veterinary clinical oncology. *Vet. Res. Commun*., v.34, Suppl.1, p.S103-S106, 2010.

OPSTAL-VAN WINDEN, A.W.; RODENBURG, W.; PENNINGS, J.L. *et al*. A bead-based multiplexed immunoassay to evaluate breast cancer biomarkers for early detection in pre-diagnostic serum. Int. J. Mol. Sci., v.13, p.13587-13604, 2012.

OWEN, L.N. *TNM classification of tumors in domestic animals*. Geneva: World Health Organization, 1980. 53p.

POOL, T.B.; HAGINO, N.; CAMERON, I.L. Relationship between functional castration and alpha-fetoprotein produced by hepatoma-bearing female rats. *J. Reprod. Fertil.*, v.53, p.39-44, 1978.

RASOTTO, R.; ZAPPULLI, V.; CASTAGNARO, M.; GOLDSCHMIDT, M.H. A retrospective study of those histopathologic parameters predictive of invasion of the lymphatic system by canine mammary carcinomas. *Vet. Pathol*., v.49, p.330-340, 2012.

REN, F.; WENG, W.; ZHANG, Q. *et al*. Clinicopathological features and prognosis of AFP-producing colorectal cancer: a single-center analysis of 20 cases. *Cancer Manag. Res*., v.11, p.4557-4567, 2019.

RICHARDSON, B.E.; HULKA, B.S.; PECK, J.L. *et al*. Levels of maternal serum alpha-fetoprotein (AFP) in pregnant women and subsequent breast cancer risk. *Am. J. Epidemiol.*, v.148, p.719-727, 1998.

SARCIONE, E.J.; BIDDLE, W. Elevated serum alpha fetoprotein levels in postmenopausal women with primary breast carcinoma. *Dis. Markers*, v.5, p.75-79, 1987.

SENHORELLO, I.L.S.; TERRA, E.M.; SUEIRO, F.A.R. *et al*. Clinical value of carcinoembryonic antigen in mammary neoplasms of bitches. *Vet. Comp. Oncol.*, v.18, p.315-323, 2020.

SORENMO K. Canine mammary gland tumors. *Vet. Clin. North Am. Small Anim. Pract*., v.33, p.573-596, 2003.

UEHARA, M.; KINOSHITA, T.; HOJO, T. *et al*. Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer. *Int. J. Clin. Oncol*., v.13, p.447-451, 2008.

URIEL, J.; BOUILLON, D.; AUSSEL, C.; DUPIERS, M. Alpha-fetoprotein: the major high-affinity estrogen binder in rat uterine cytosols. *Proc. Natl. Acad. Sci. U.S.A*., v.73, p.1452-1456, 1976.

UVA, P.; AURISICCHIO, L.; WATTERS, J. *et al*. Comparative expression pathway analysis of human and canine mammary tumors. *BMC Genomics*, v.10, p.135, 2009.

VILLACAMPA, M.J.; MORO, R.; NAVAL, J. *et al*. Alpha-fetoprotein receptors in a human breast cancer cell line. *Biochem. Biophys. Res. Commun*.,v.122, p.1322-1327, 1984.

WANG, G.; QIN, Y.; ZHANG, J. *et al*. Nipple discharge of CA15-3, CA125, CEA and TSGF as a new biomarker panel for breast cancer. *Int. J. Mol. Sci*., v.15, p.9546-9565, 2014.

YAMADA, T.; FUJITA, M.; KITAO, S. *et al*. Serum alpha-fetoprotein values in dogs with various hepatic diseases. *J. Vet. Med. Sci*., v.61, p.657-659, 1999.

YAMAGAMI, T.; KOBAYASHI, T.; TAKAHASHI, K.; SUGIYAMA, M. Prognosis for canine malignant mammary tumors based on TNM and histologic classification. *J. Vet. Med. Sc*i.,v.58, p.1079-1083, 1996.

YANG, N.Y.; ZHENG, H.H.; YU, C. *et al*. Research progress of good markers for canine mammary carcinoma. *Mol. Biol. Rep*., v.50, p.10617-10625, 2023.