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The prognostic value of serum neoplastic biomarkers CA 15-3 and CEA in canine mammary neoplasms: a review

O valor prognóstico dos biomarcadores neoplásicos séricos CA 15-3 e CEA em neoplasias mamárias caninas: uma revisão

Breno Queiroz Pinheiro, Isaac Neto Goes da Silva[2], Augusto Manuel Rodrigues Faustino[3], Lúcia Daniel Machado da Silva[1]

[1] Laboratory of Carnivore Reproduction, School of Veterinary Medicine, State University of Ceará, 60714-903, Fortaleza, Brazil
[2] Laboratory of Veterinary Clinical Pathology, School of Veterinary Medicine, State University of Ceará, 60714-903, Fortaleza, Brazil
[3] Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences of Abel Salazar, University of Porto, 4050-313, Porto, Portugal

Resumo

As neoplasias mamárias caninas (NMCs) são as mais frequentes em cadelas, com isso, uma grande variedade de técnicas tem sido utilizada para identificar os seus fatores prognósticos. Dentre as possibilidades, os biomarcadores neoplásicos séricos antígeno do câncer (CA 15-3) e o antígeno carcinoembrionário (CEA) são os mais promissores. Biomarcadores neoplásicos são substâncias presentes na neoplasia, sangue ou demais produtos biológicos, produzidos pela neoplasia, ou secundariamente pelo paciente, em resposta à sua presença. Na medicina veterinária, esses marcadores são pouco estudados. Em termos gerais, eles não podem ser usados para o diagnóstico primário de neoplasias mamárias, mas estão relacionados com os fatores prognóstico já bem estabelecidos na literatura e tem como vantagem se tratar de uma análise menos invasiva e que é possível de ser feita de forma seriada. O objetivo desta revisão é descrever o CA 15-3 e o CEA como biomarcadores utilizados nas NMCs, o progresso recente feito na literatura e providenciar um sumário dos principais resultados já obtidos. O CA 15-3 e o CEA apresentam potencial prognóstico nas NMCs. Apesar de existir uma grande variação de resultados para esses biomarcadores na literatura, o seu uso deve ser considerado, visto os resultados obtidos na caracterização e prognóstico das NMC.

Palavras-chave: antígeno do câncer, antígeno carcinoembrionário, biomarcador tumoral, cão, tumor mamário.

Abstract

Female canine mammary neoplasms (CMNs) are the most frequent neoplasm in bitches, and a variety of techniques have been used to identify they prognostic factors. Among the possibilities, the serum biomarkers cancer antigen (CA 15-3) and carcinoembryonic antigen (CEA) are the most reliable. Neoplastic biomarkers are substances present in the neoplasia, blood, or other biological products, produced mainly by the neoplasia, or secondarily by the patient, in response to their presence. In veterinary medicine, these biomarkers are poorly studied. In general terms they cannot be used for primary diagnosis of mammary neoplasms but are related to the prognostic factors and have the advantage of being a less invasive analysis and that it is possible to be done in a serial way. The objective of this review is to describe CA 15-3 and CEA use as biomarkers in CMNs, the recent progress made in literature and the main overall results that had been already obtained. CA 15-3 and CEA have prognostic potential for CMNs. There is a wide variation of results for these biomarkers in literature, but despite this, its use must be considered as they provide relevant results in terms of characterization and prognostic in CMNs.

Keywords: biomarker tumor, cancer antigen, carcinoembryonic antigen, dog, mammary tumors.

Introduction

Female canine mammary neoplasms (CMNs) are the most common neoplasm in dogs, representing 50% to 70% off all neoplasms in this subset of population, and is a complex and heterogenous

[1]Correspondência: breno.queiroz@uece.br
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disease with difficulties in its classification, diagnosis, and prognosis (Cassali et al., 2020; Sorenmo, 2020). Studies suggest the annual incidence rate (IR) of CMNs was 111 to 250 cases per 100,000 dogs (Dobson et al., 2002; Egenvall et al., 2005; Vascellari et al., 2020). These incidences are increasing, and annual incidence rate now are comparable to that in women (Vascellari et al., 2020).

A variety of techniques have been used to identify new prognostic factors in CMNs (Webster et al., 2011; Kaszak et al., 2018; Cassali et al., 2020). Among then, there are the serum biomarkers cancer antigen (CA 15-3), and carcinoembryonic antigen (CEA) are the most reliable (Mobasheri et al., 2010).

Campos et al. (2012) performed Western blotting analysis to confirm the specificity and possible cross-reactivity of human CA 15-3 and CEA antibodies with canine serum and indicated a good interaction between human and canine CA 15-3 and CEA antibodies. Besides Manuali et al. (2012) demonstrated a high similarity in the DF3 epitope of the Mucin 1 (MUC1), the target detected in the assays of CA 15-3, in human and dogs. This suggests that the determination of these biomarkers may be considered in bitches, using the same reagents kits used in humans.

Is known that is possible to use chemiluminescent (Marchesi et al., 2007; Marchesi et al., 2010; Manuali et al., 2012; Campos et al., 2012), radioimmunoassay (RIA) (Valencakova-Agyagosova et al., 2012) and Enzyme-linked immunosorbent assay (ELISA) technology (Campos et al., 2015; Senhorello et al., 2019; Fan et al., 2021; Jain et al., 2021; Ramadan et al., 2021) for the determination of CA 15-3 and CEA in clinically healthy bitches and with CMNs (Table 1). The latter is the most common technique used for the detection in serum (Li et al., 2019).

In cases of CMNs only have been published few papers evaluating the prognostic potential of CA 15-3 and CEA, with small and/or very heterogeneous groups of patients, using different molecular techniques, and a short follow up period (Marchesi et al., 2007; Marchesi et al., 2010; Manuali et al., 2012; Campos et al., 2012; Valencakova-Agyagosova et al., 2012; Campos et al., 2015; Roberto et al., 2018; Baba et al., 2019; Senhorello et al., 2019; Fan et al., 2021; Jain et al., 2021; Ramadan et al., 2021) (Table 1). None of these studies aimed to evaluate the effectiveness of these biomarkers in primary diagnosis.

In this light, the objective of this review is to summarize and described the prognostic value of serum neoplastic biomarkers CA 15-3 and CEA in CMNs, the recent progress made in literature with main overall results and some recommendations for clinical use of these biomarkers.

Cancer antigen 15-3 (CA 15-3)

After the hybridoma technology developed by Köhler and Milstein (1975) becomes a routine tool, breast neoplasm cells were used to immunize BALB/c mice to generate monoclonal antibodies against neoplasm cell-associated antigens (Kufe et al., 1984). Two such monoclonal antibodies, DF3 and 115D8, were used to develop an immunoassay, which is subsequently used to detect a specific cancer antigen named CA 15-3, CA15/3 or CA153 in the sera of breast neoplasm patients (Tondini et al., 1988).

Further studies showed that the monoclonal antibody DF3 recognizes the core protein of Mucin 1 (MUC1) (Abe e Kufe, 1989) whereas the monoclonal antibody 115D8 recognizes part of the glycan chains on MUC1 (DAI et al., 1998). The DF3 is an IgG1 whereas the 115D8 is an IgG2b-k with the apparent affinities of 5.26 X 10^{-9} and 1.84 X 10^{-9} M, respectively, to MUC1 (Dai et al., 1998).

In humans and mice, MUC1 is normally expressed in the glandular or luminal epithelial cells of the mammary gland, esophagus, stomach, duodenum, pancreas, uterus, prostate, and lungs, and to a lesser extent, in hematopoietic cells (Gendler, 2001). In healthy tissues, MUC1 provides protection to the underlying epithelia creating a physical barrier limiting accessibility and preventing pathogenic colonization (Yolken and Peterson, 1992).

Most neoplasic cells present the transmembrane glycoprotein antigen MUC1 hypoglycosylated, redistributed over the cell surface and within the cytoplasm leading to a lack of cell polarity of the epithelial cells, these facts act as anti-adhesive mechanism and facilitating the detachment of malignant cells, and enabling MUC1 to be measured in serum (Nath and Mukherjee, 2014).

The importance of MUC1 in disease progression are related to neoplasm invasion, metastasis, expression of proangiogenic factors, production of growth factors, blocks hypoxia-induced cell death by mediating decreases in intracellular ROS, drug resistance by preventing the activation of the intrinsic apoptotic path-way and synthesis of IL-6 and TNF-a creating a proinflammatory milieu in the neoplasm microenvironment (Cheung et al., 2000; Hollinghsworth e Swanson, 2004; Cascio et al., 2011; Nath and Mukherjee, 2014; David et al., 2016).

CA 15-3 in canine mammary neoplasms

In CMNs cases is reported a significant increase in tissue membrane immunostaining of MUC1 compared to healthy ones (Manuali et al., 2012). This allows MUC1 to be shed into the circulation where it can be measured by immunoassays as CA 15-3 (Hollinghsworth and Swanson, 2004; David et al., 2016).

Valencakova-Agyagosova (2012) proposes the cut-off value of 5.0 to 7.0 IU/mL in cases of CMNs, which are very similar that the cutt-off reported by Jain et al. (2021) of 5.65 IU/mL. These results differ from the cut-off of 0,50 IU/mL proposed by Campos (2015), this difference might have occurred mainly because of different methodologies used, differences in clinical stage and histological grades of the patients in these works (Table 1). Until now, there are no reference intervals for CA 15-3 in the serum of normal bitches, a fact that that makes further studies necessary.

Most studies show a statistical difference in serum CA 15-3 concentration between groups with and without CMNs (Marchesi et al., 2010; Manuali et al., 2012; Campos et al., 2012; Valencakova-Agyagosova et al., 2012; Campos et al., 2015; Baba et al., 2019; Fan et al., 2021; Jain et al., 2021; Ramadan et al., 2021). Roberto et al. (2018), despite finding differences in CA 15-3 serum dosages between canine females with and without CMNs, they demonstrated that the serum levels of CA 15-3 between patients with benign and malignant neoplasms were not significantly different, but most recently Fan et al. (2021) and Jain et al. (2021) also subdivide the mammary neoplasm group in benign and malign and reported a statistical difference between them.

When compared the serum concentrations with the clinical pathology parameters there is a significantly higher concentration of CA 15-3 in bigger neoplasms (Campos et al., 2012; Campos et al., 2015; Jain et al., 2021), lymph node metastasis (Campos et al., 2012; Campos et al., 2015; Fan et al., 2021), higher histopathological grades (Manuali et al., 2012; Fan et al., 2021) and distant metastasis (Fan et al., 2021) (Table 2). Valencakova-Agyagosova (2012) hypothesized that reproductive status can have possible effect on CA 15-3, but Roberto (2018) observed that the animals' estrous cycle did not influence the values of the biomarker in canine females with CMNs.

The serum CA 15-3 concentration decreased significant post-surgery which may be good prognostic indicators and suggesting its possible use as a neoplasm recurrence control after mastectomy (Roberto et al*.,* 2018). Baba et al. (2019) despite not finding differences in the post-surgical period, found a lower mean survival in patients with higher serum concentrations of CA 15-3.

Carcinoembryonic antigen (CEA)

CEA is one of the best-known human neoplastic biomarkers discovered by Gold and Freedman in 1965 from colon neoplasm. They used an antibody obtained from a mouse previously immunized with an extract from hepatic metastases of an intestinal carcinoma. This antibody is produced by the induction of a surface glycoprotein involved in intracellular adhesion that contains 45 to 50% carbohydrates. The structure of this glycoprotein consists of a polypeptide chain consisting of 641 aminoacids, with lysine at its N-terminal position that has approximately 200 kD molecular weight (Grunnet and Sorensen, 2012).

CEA it is produced, mostly, by gastrointestinal mucosa, localized in epithelial cell membranes in small amounts, and it is overexpressed by epithelial cells of the colon, breast, and lungs (Kaszak et al., 2018).

CEA has less sensitivity and specificity compared with CA 15-3 and for that is recommended as a biomarker of second option, subsidiary biomarker, which provides generally support to the main biomarker, the CA 15-3, and increased the detection of neoplasms (Valencakova-Agyagosova et al., 2012).

CEA in canine mammary neoplasm

In dogs with mammary gland neoplasms, serum levels of CEA were elevated compared to healthy dogs (Valencakova-Agyagosova et al., 2012; Baba et al., 2019; Senhorello et al., 2019; Jain et al., 2021).

Table 1. Manly results in literature for concentrations of CA 15-3 and CEA in serum of control bitches and with CMNs.

Authors	N	C	CMNs	Methodology	CEA (C) ng/mL	CA 15-3 (C) IU/mL	CEA (CMNs) ng/mL	CA 15-3 (CMNs) IU/mL
Marchesi et al., 2007	105	30/31	44	Chemiluminescent	*	3 ± 0.14	*	2.73 ± 0.17**
Marchesi et al., 2010	81	24	57	Chemiluminescent	*	0.57 ± 0.21	*	0.79 ± 0.56
Manuali et al., 2012	50	*	50	Chemiluminescent	*	0.57 ± 0.21	*	0.80 ± 0.55
Campos et al., 2012	90	30	60	Chemiluminescent	0.19 ± 0.20	1.19 ± 0.51	II (40) = 0.12 ± 0.12/ III (12) = 0.29 ± 0.36/ IV (8) = 0.07 ± 0.04	II (40) = 1.61 ± 0.61/ III (12) = 2.39 ± 1.02/ IV (8) = 2.46 ± 1.00
Valencakova-Agyagosova et al., 2012	45	20	25	Radioimmunoassay	0.20 ± 0.03	5.14 ± 1.34	0.25 ± 0.06	8.58 ± 1.27
Campos et al., 2015	48	20	28	ELISA	*	0.31 ± 0.19	*	II (14) = 0.53 ± 0.45/ III (14) = 1.08 ± 0.44
Roberto et al., 2018	40	40	40	Chemiluminescent	*	0.19 ± 0.39	*	1.56 ± 0.39
Baba et al., 2019	47	6	41	ELISA	1.03 ± 0.17	1.25 ± 0.04	1.84 ± 0.09	1.33 ± 0.01
Senhorello et al., 2019	77	21	56	ELISA	0.60 ± 0.34	*	II (31) = 1.50 ± 0.71/ III (12) = 2.19 ± 0.77/ IV (13) = 2.76 ± 0.73	*
Fan et al., 2021	178	40	138	ELISA	*	*	*	*
Jain et al., 2021	60	20	40	ELISA	201.03 ± 48.54	B = 281.08 ± 83.75/ M = 377.92 ± 65.80c	5,02 ± 0,90	B = 5,91 ± 0,60/ M = 7,71 ± 0,88
Ramadan et al., 2021	17	7	10	ELISA	0.14 ± 0.03	0.15 ± 0.05	1,33 ± 0,10	3,76 ± 0,43

N = Number of animals. C = Number of animals in the control group. * Not reported. ** Not only dogs with CMNs were evaluated. Campos et al., 2012: II (T1,2,3N0M0); III (T1,2,3N1,2M0); IV (T1,2,3N1,2M1). Campos et al., 2015: II (T1,2,3N0M0); III (T1,2,3N1,2M0). Senhorello et al., 2019: II (T1N0M0); III (T2.3N0M0); IV (T1,2,3N1,2M0). Jain et al., 2021: B = Benign breast neoplasms; M = Malignant breast neoplasms.

Table 2. Main related results in literature of CA 15-3 and CEA with the prognostic factors in CMNs.

Authors	Biomarker	Statistical difference	Cut-off	Size related	Grade related	Lymph node metastasis related	Decreasing post-surgery
Marchesi et al., 2007	CEA	*	**	**	**	**	**
	CA 15-3	No	**	**	**	**	**
Marchesi et al., 2010	CA 15-3	Yes	**	**	**	**	**
Manuali et al.,2012	CA 15-3	Yes	**	No	Yes	**	**
Campos et al., 2012	CEA	No	**	**	**	**	**
	CA 15-3	Yes	**	Yes	**	Yes	**
Valencakova-Agyagosova et al., 2012	CEA	Yes	0.20–0.23 ng/mL	**	**	**	**
	CA 15-3	Yes	5.0–7.0 IU/mL	**	**	**	**
Campos et al., 2015	CA 15-3	Yes	0.50 IU/mL	Yes	**	Yes	**
Roberto et al., 2018	CA 15-3	Yes	**	**	**	**	Yes
Baba et al., 2019	CEA	Yes	**	**	**	**	Yes
	CA 15-3	Yes	**	**	**	**	Yes
Senhorello et al., 2019	CEA	Yes	1.08 ng/mL	Yes	Yes	Yes	Yes
Fan et al., 2021	CEA	No	**	**	Yes	Yes	**
	CA 15-3	Yes	**	**	Yes	Yes	**
Jain et al., 2021	CEA	Yes	247.65 ng/L	**	**	**	**
	CA 15-3	Yes	5.65 IU/mL	Yes	**	**	**
Ramadan et al., 2021	CEA	No	**	**	**	**	**
	CA 15-3	Yes	**	**	**	**	**

* Not able to be measured. ** Unreported.

CEA serum values in canine females without CMNs is approximately ten times smaller than in humans and ranged from 0.00 to 0.23 ng/mL (Campos et al., 2012).

As observed for CA 15-3, there is a wide variation of results for CEA, and once more this difference mainly occurred probably because of different methodologies used and the use of different reagents (Table 1) (Campos et al., 2012; Valencakova-Agyagosova et al., 2012; Baba et al., 2019; Senhorello et al., 2019; Fan et al.. 2021; Jain et al., 2021; Ramadan et al., 2021).

CEA is a neither specific nor sensitive biomarker for primary diagnosis of CMNs but can be a subsidiary biomarker to detect CMNs (Jain et al., 2021). CEA level is not aimed only at diagnosis but also for assigning a prognosis and follow-up, to detect recurrence in patients who have undergone surgery, and to monitor the therapeutic response (Valencakova-Agyagosova et al., 2012; Baba et al., 2019; Senhorello et al., 2019) (Table 2).

Senhorello et al. (2019) propose the CEA cut-off value of 1.08 ng/mL for malignant CMNs detection, which is very discrepant of the result found by Jain et al. (2021) of 247.65 ng/L. This difference can be explained by the same justification used in CA 15-3.

The serum CEA concentration was found to be lowest in healthy bitches, higher in bitches with benign neoplasms, and highest in bitches with malignant CMNs (Jain et al., 2021). The concentration of CEA was also higher in bigger neoplasms (Senhorello et al., 2019), lymph node metastasis (Senhorello et al., 2019; Fan et al. 2021), higher histopathological grades (Senhorello et al., 2019; Fan et al. 2021) and distant metastasis (Fan et al., 2021) (Table 2).

CEA values started decreasing 15 days after the neoplasm was removed, which may be a good prognostic indicator, but no significant difference was observed when compared with the presurgical levels (Baba et al., 2019; Senhorello et al., 2019), suggesting that further studies aiming at greater follow-up should be performed.

Recommendations for clinical use in CMNs

Since these biomarkers are relatively easy, less invasive, and cheap to determine compared with immunohistochemical tests, preoperative levels might be combined with existing prognostic factors in planning the optimum CMNs management. Even after mastectomy we recommend serial levels measurements of these serum biomarkers during the follow-up, particularly of asymptomatic patients, as increases in the concentration should be related with a worst outcome, and in this case additional procedures must be performed. Also, the sensitivity and accuracy of the combined detection of these neoplastic biomarkers are significantly higher than that of single detection.

Concluding remarks

Human kits for CA 15-3 and CEA have proven to be useful in bitches with CMNs. CA 15-3 and CEA cannot be used for screening the general asymptomatic population or for independently diagnosing in cases of CMNs, therefore they have been proven to be correlated to the prognostic factors and could play an important role in the management and follow-up of patients. Most of the studies in literature regarding CA 15-3 and CEA in CMNs evaluate only a small group of patients and/or very heterogeneous groups and use different molecular techniques. Looking into the future, more detailed studies should be carried out with a large prospective randomized trial and with the emphasis moving from single to multiple biomarkers, especially in screening for early disease, and in focusing on a longer follow-up evaluation.

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