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EXTENDED ABSTRACT

Cancer antigen 15/3: possible diagnostic use in veterinary clinical oncology. Preliminary study

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Abstract The cancer antigen 15/3 is a mucin that is associated with the cell membrane, encoded by the MUC1 gene, and recognized by the monoclonal-clone DF3 antibody. The latter antigen was discovered to be specific for both the identification of human mammary neoplasia and during patient follow-up evaluations. The aim of this study is to report and compare the results of the application of direct chemiluminescence in canine blood sera and the kit utilized in human medicine for the determination of Ca 15/3 to verify the diagnostic efficiency of the kit in cases presenting mammary tumors. Specifically, CA 15/3 has proven to be measurable in all samples assayed to distinguish clinically healthy subjects from those with mammary neoplasia.

Keywords CA15/3 · Serum · Dog · Mammary tumor

Introduction

Circulating markers are glycoprotein substances that can be measured in bodily fluids from both healthy and neoplastic tissues; in the latter, they are usually produced in higher quantities and function as indicators of tumour presence (Bombardieri 1987). The cancer antigen 15/3 is a high molecular weight mucine, encoded by the *MUC1* gene (Stieber et al. 1999; Hayes et al. 1986), associated with cell membranes, and recognized by the monoclonal antibody DF3. This marker has been shown to be specific for human mammary neoplasia as well as for the monitoring of tumour behaviour in response to treatment.

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Specifically, it has been demonstrated that elevated, pre-operative levels of this glycoprotein in blood serum is correlated with negative prognostic significance, including tumour dimensions, stage of disease, and presence of metastasis (Safi et al. 1991; Molina et al. 2003; Martin et al. 2006; Al-Azawi et al. 2006). Considering that CA 15/3 is a mucinic marker, serum levels can reflect increased broncho-pulmonary and gastroenteric carcinomas (Ruibal et al. 1986; Schimdt-Rhode et al. 1987); however, increased levels of CA 15/3 can also be found in inflammatory pathologies (Colomer et al. 1989; Valerio Marzano et al. 1998). In female dogs, mammary gland neoplasia represents one of the most frequently observed tumours (Misdorp 2002) of which the prevalence of malignant lesions varies from 26% to 73% (Perez-Alenza et al. 2000); however, higher percentiles have been reported in the literature (Karayannopoulou et al. 1990; Karayannopoulou et al. 2003). Therefore, it would be very useful to have an early indicator of mammary neoplastic disease in the field of veterinary clinical oncology. In this study, the reported results are based on a broader research project with the collaboration and participation of the Internal Medicine Section of the Pathology, Diagnostic and Veterinary Clinic, Department of the Experimental Zooprophylactic Institute, and the Hematology and Clinical Service, Hospital of Perugia. This study aimed to enrich the "Registry of Animal Tumours" and to consider the behaviour of canine mammary neoplasia as a model of human mammary neoplasia. Furthermore, we reported data relative to the determination of CA 15/3 in the dog, which was done to evaluate the presence of such a tissue marker, to identify a sensitive and applicable method to assay CA 15/3 in dog sera, and to verify its diagnostic significance during the course of mammary tumorigenesis.

Materials and methods

In this study, we used 57 serum and tissue samples of female dogs with mammary neoplastic lesions (Group B) as well as 24 serum samples of clinically healthy subjects, considered the control group (Group A). Serum was stocked at −20°C and processed for the determination of CA 15/3 by direct chemiluminescence method (ADVIA Centaur) using a kit for human diagnostic oncology (Bayer Immuno 1 CA 15-3). The values obtained were expressed in U/mL. The portions of mammary tissue that were derived from subjects within the age range of 3–17 years (9.09±2.4), belonging to both selected and mixed breeds, were formaldehyde-fixed and embedded in paraffin using standard procedures. Serial sections of 4 μm in thickness were stained with hematoxylin-eosin (HE), the lesions were classified using the diagnostic criteria proposed by Misdorp (Misdorp et al. 1999), and the presence of possible inflammation was evaluated. Ultimately, to demonstrate tissue expression of CA 15/3, immunohistochemical labelling was performed using the monoclonal antibody anti-human CA 15/3, clone DF3, using the streptavidin-peroxidase procedure (DakoCytomation, Denmark). The results of this CA 15/3 determination were statistically analyzed using ANOVA ($p \leq 0.05$) as well as a beta distribution of the probability of tumour presence.

Results

The serum CA 15/3 concentrations (mean values ± S.D.) were 0.79±0.56 U/mL in female dogs affected with neoplasia (Group B) and 0.57±0.21 U/mL in clinically healthy subjects (Group A). The beta distribution of the probability of tumour presence is represented in Fig. 1. The histological examination, which allowed for the categorization of subjects in the

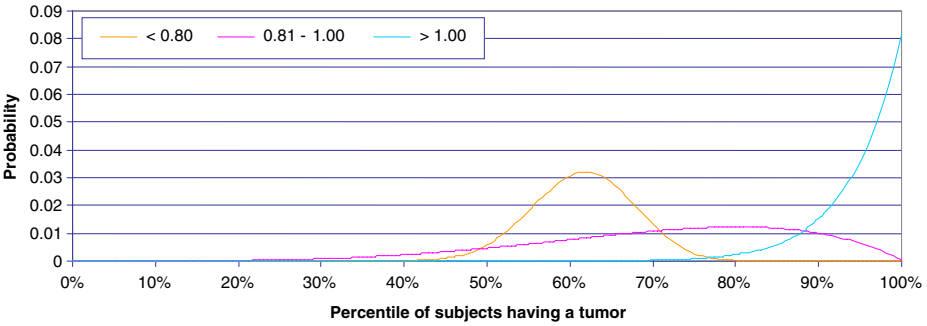

Fig. 1 Distribution of tumour presence probability

B group, revealed 50 malignant neoplasias (78.2%) and seven benign neoplasias (21.8%). Of the tumour-carrying subjects, 84.4% were intact females, whereas 15.2% were spayed females. The histotypes that were more frequently encountered were complex carcinomas (59.7%), simple carcinomas (21.1%), and complex adenomas (8.8%); we also performed diagnoses of solid carcinomas (3.5%), carcinosarcomas (3.5%), simple adenomas (1.7%), and mixed benign tumours (1.7%). Immunohistochemistry revealed the presence of the tumour marker in 73.7% of cases.

Discussion

Based on our experience, we show that mammary tumours are of greater incidence in intact female dogs (84.8%) in agreement with previous studies (Stieber et al. 1999). Furthermore, the malignant forms encountered make up 78.2% of all neoplastic forms. This elevated percentile could be correlated with the fact that the animal is typically brought to a surgeon when the stage of neoplastic disease is already advanced, and, thus, the tissue samples that were sent for histological examination demonstrated signs of malignancy. Direct chemiluminescence confirmed this finding using a kit to identify human antigen CA 15/3 in all processed sera. The results of the CA 15/3 determination performed on group A, expressed in mean values ± standard deviation, were considered reference limits, identifying a correlation of the marker between healthy animals and animals with neoplasia. Specifically, we showed a statistically significant difference (p=0.04) between the assayed values in the two groups. The elevated standard deviation of the values of CA 15/3 in group B can be explained from the variability of tumour morphology. Group B possessed both benign and malignant neoplasias, and within the latter, there were different histotypes, with different prognostic values not classifiable into homogeneous sub-groups. Moreover, from the beta distribution of tumour presence probabilities, we show in Fig. 1 that neoplastic mammary pathology is found in subjects with serum CA 15/3 levels higher than 1 U/mL. Furthermore, since the inflammatory process is often associated with neoplasia, it does not appear to have influenced the levels of CA 15/3 in serum as neoplastic samples associated with inflammation had CA 15/3 levels of 0.6±0.4 U/mL, and in healthy samples, they were 0.9±0.6 U/mL. Ultimately, we find it interesting and worth mentioning how immunohistochemical analyses targeted to the DF3 clone in proliferating epithelial cells demonstrated tissue expression of this marker in canines, resulting in 73.7% of the diagnosed tumours. Even though our data need to be considered preliminary and, thus, more research is necessary to evaluate the sensitivity and specificity of the method, the use of direct

chemiluminescence and kits used in human medicine appears to be promising for the determination of CA 15/3 levels in canine mammary oncology diagnostics. It is our intention to increase the study population to overcome these limits, which are explained by the ample variety of tumour histotypes, and to be able to evaluate the utility of the determination of CA 15/3, not only for identification of neoplastic diseases, but also to monitor patients during follow-up.

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