VETERINARSKI ARHIV 81 (6), 709-722, 2011

Histopathological evaluation and immunohistochemical study of estrogen receptor α, HER-2 and Ki-67 in canine neoplastic mammary lesions

Andrea Gudan Kurilj[1]*, Marko Hohšteter[1], Branka Artuković[1], Krešimir Severin[2], Ivan-Conrado Šoštarić-Zuckermann[1], Ana Beck[1], Sven Seiwerth[3], Ruža Sabočanec[1], and Željko Grabarević[1]

[1]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Department of Forensic Veterinary Medicine, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[3]*Institute of Pathology, School of Medicine, University of Zagreb, Zagreb, Croatia*

GUDAN KURILJ, A., M. HOHŠTETER, B. ARTUKOVIĆ, K. SEVERIN, I.-C. ŠOŠTARIĆ-ZUCKERMANN, A. BECK, S. SEIWERTH, R. SABOČANEC, Ž. GRABAREVIĆ: Histopathological evaluation and immunohistochemical study of estrogen receptor α, HER-2 and Ki-67 in canine neoplastic mammary lesions. Vet. arhiv 81, 709-722, 2011.

ABSTRACT

The frequency of different types of mammary lesions and their relationship with histologic grade was investigated. One hundred and forty-six mammary lesions were classified according to the World Health Organization (WHO) criteria. Selected lesions (51 malignant and 24 benign) were investigated to determine the immunohistochemical expression of estrogen receptor alpha (ERα), HER-2 and Ki-67 and their relationship with tumor factors. The most common breeds affected were cross breeds, poodles, cocker spaniels and German shepherds and the median age of tumor diagnosis was 10 years (range 4 - 15 years of age). Classification of all canine mammary gland lesions revealed 110 (75.3%) malignant and 36 (24.7%) benign tumors. ERα was expressed by 9/24 (37.5%) benign and 9/51 (17.6%) malignant tumors. HER-2 protein was detected in 5/51 (9.8%) malignant tumors but none in the 24 benign tumors. The Ki-67 index was higher in malignant (mean 29.5) than benign (mean 9.1) tumors ($P<0.001$) and significantly higher in anaplastic carcinomas than tubulopapillary carcinomas ($P<0.05$).

Key words: mammary tumors, dog, ERα, HER-2, Ki-67

*Corresponding author:

Andrea Gudan Kurilj, DVM., PhD, Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Heinzelova 55, Croatia, Phone: +385 1 2390 314; E-mail: agudan@vef.hr

ISSN 0372-5480
Printed in Croatia

Introduction

Mammary tumors are among the most prevalent neoplasms in female dogs (LANA et al., 2007) and occur mostly in countries where dogs are not routinely spayed at an early age (SORENMO et al., 2000). The average age at first detection is 10 - 11 years (GINN et al., 2007). According to the histologic diagnosis, between 41 and 53% of the mammary tumors that occur in bitches are considered to be malignant (LANA et al., 2007). Canine mammary tumors (CMT) are heterogeneous in their pathological features and clinical behavior (NIETO et al., 2000). Therefore, reliable prognostic factors are of great importance for estimating the individual risk of unfavorable clinical outcome. There are some recognized, well-accepted prognostic factors of malignant mammary tumors in dogs, such as tumor size, lymph node status, distant metastasis, histologic type, histologic malignancy grade, degree of nuclear differentiation and proliferative activity (BRATULIĆ et al., 1996; MISDORP et al., 1999; MISDORP, 2002; MARTIN de las MULAS et al., 2005), and also, the increasing availability of antibodies for immunohistochemical studies has allowed additional criteria to be evaluated (ZAIDAN DAGLI, 2008; MORRIS, 2010).

Estrogen receptors (ER) are expressed by more than 50% of canine mammary gland tumors (MARTIN de las MULAS et al., 2005; YANG et al., 2006; LANA et al., 2007), implying that reproductive hormones play an important role in the initiation and progression of mammary tumors, which is also supported by the protective effects of ovariectomy (SORENMO et al., 2000) and the sporadic occurrence of mammary dysplastic and neoplastic lesions in animals treated with progestagens (PEREZ ALENZA et al., 2000). Normal canine mammary glands, dysplasia and benign tumors differ from malignant tumors in expressing higher levels of ERα, and tumors that metastasize are frequently negative for ERα (NIETO et al., 2000; MILLANTA et al., 2005; MARTIN de las MULAS et al., 2005; YANG et al., 2006).

HER-2 (also known as *neu*, *HER-2*/*neu* and *c-erb*B-2) is a cell membrane receptor with an intracellular protein kinase domain and it belongs to a family of membrane-bound protein kinases that include an epidermal growth factor receptor (EGFR) (KILLEEN, 2004). Overexpression of HER-2 is found in 20 - 30% of human breast carcinomas and such patients have shorter survival time and generally a poor prognosis (ROSS and FLETCHER, 1999; SAHIN, 2000; LESTER, 2010). This oncogene has also been explored as prognostic marker in CMT and studies showed that either HER-2 amplification (AHERN et al., 1996) or HER-2 protein overexpression (RUNGSIPIPAT et al. 1999; MARTIN de las MULAS et al., 2003; HSU et al., 2009) were present in canine mammary tumors. One study using chromogenic in situ hybridization (CISH) indicated that gene amplification was not present in canine mammary carcinomas (MARTIN de las MULAS et al., 2003), and another study demonstrated that dogs with HER-2 - overexpressing malignant mammary tumors tended to have a higher survival rate than those that expressed a normal level of HER-2 (HSU et al., 2009).

Neoplasms are characterized by an excessive and uncoordinated proliferation of cells (MISDORP, 2002). Studies on canine mammary tumors have revealed an association between proliferative activity and several clinical and pathological variables, such as histologic grade, nuclear pleomorphism, absence of hormonal receptors, metastasis and survival rate (PEREZ ALENZA et al., 1995; PENA et al., 1998; GERALDES et al., 2000). The Ki-67 nuclear antigen is one of the most commonly used immunohistochemical markers to evaluate tumor proliferative activity. It is expressed in all active phases of the cell cycle (G1, S, G2, M) but not in quiescent cells (G0) (GERDES et al., 1984). In canine mammary tumors, Ki-67 showed increased percentages of positive cellular staining with the increasing malignancy of the histologic type (GERALDES et al., 2000; MUTO et al., 2000; YANG et al., 2006; MORRIS et al., 2009).

The present study was undertaken to determine the relative frequencies of different tumor types and the histologic malignancy grade (HMG) of mammary gland tumors in a population of female dogs in Croatia, and to investigate the expression of ERα, HER-2 and Ki67 in dysplastic and neoplastic canine mammary tissue and their relationship with histologic grade.

Materials and methods

Histologic examination. Surgical specimens of canine mammary tissue submitted to the Department of veterinary pathology, University of Zagreb, from 2005 to 2007 for diagnostic purposes were selected for review. The majority of specimens were from animals surgically treated at the Clinics of the Veterinary Faculty, and a smaller number of specimens were from private practices all around Croatia. Samples were fixed in 10% neutral buffered formalin, and some samples were delivered already formalin fixed. Samples were then embedded in paraffin wax and 2 μm sections were stained with hematoxylin and eosin (H&E) for routine histopathological examination.

Classification and grading of lesions. H&E stained sections of canine mammary tissue were classified by two different pathologists, according to the diagnostic criteria proposed by the WHO (MISDORP et al., 1999). The histologic malignancy grade of the carcinomas was assessed according to MISDORP (2002), by examining tubule formation, intensity of nuclear staining and mitoses and nuclear pleomorphism. A score of 1 to 3 was given for each feature, and to obtain the overall tumor grade, the scores for each factor are added together, giving a possible total of 3-9 pints. Tumor grade could then be allocated on the following basis: 3-5 points grade I (well differentiated tumors), 6-7 points grade II (moderately differentiated tumors) and 8-9 points grade III (poorly differentiated tumors).

Immunohistochemistry. Seventy-five samples were selected (on the basis of the quality of tissue available) for immunohistochemical analysis, and assays were performed

on 2 μm sections of paraffin-embedded tissue samples. The sections were dewaxed in xylene and rehydrated through a series of graded alcohol solutions. Antigen retrieval was carried out by microwave treatment with ethylenediaminetetraacetic acid (EDTA) buffer, pH 9 (DakoCytomation, Code S2367) for ERα 4x5 min, for HER-2 3x5 min, and for Ki-67 with citrate buffer pH 6 (DakoCytomation, Code S2031) for 20 min. Endogenous peroxidase activity was blocked by incubating the slides for 5 min at room temperature in Dako REAL™ peroxidase blocking solution (DakoCytomation, Code S2023). Sections were incubated with primary antibodies for 30 min using the monoclonal mouse anti-human ERα (DakoCytomation No. M7047) diluted 1:35, polyclonal rabbit anti-human c-erbB-2 (DakoCytomation No. A0485) diluted 1:350, and monoclonal mouse anti-human Ki-67 (clone MIB-1, DakoCytomation No. M7240) diluted 1:75. This was followed by incubation for 30 min with a ready-to-use secondary antibody (Dako REAL™ EnVision™/Horseradish Peroxidase, Rabbit/Mouse) and with the substrat Dako REAL™ Diaminobenzidine + Chromogen for a further 10 min. Rinses were done with DakoCytomation Wash Buffer between each step. The sections were counterstained with hematoxylin and mounted. Sections from human benign breast tumor were used as positive control for ERα, from breast carcinoma as a positive control for HER-2 and from canine intestines as a positive control for Ki-67. The substitution of the primary antibody with phosphate buffered saline was used as negative control.

Evaluation of immunohistochemical staining was executed semiquantitatively by examining the entire tumor present in the tissue section using an immunoperoxidase score. For ERα, the percentage of tumor cells with positive staining (proportion score, PS) was estimated and graded from 0 to 3. Zero points meant less than 5% staining, 1 point represented 5-19%, 2 points 20-60%, and 3 points indicated more than 60% of the tumor nuclei showed positive staining. The staining intensity score (intensity score, IS) estimated the average staining intensity of positive tumor cells, and was scored as 0 = negative, 1 = faint, 2 = moderate staining, 3 = strong staining. The PS and IS were added to obtain a total score (TS) (range 0-6). ERα was considered positive when PS was ≥ 1 and TS ≥ 2 as previously described (MARTIN de las MULAS et al., 2005; YANG et al., 2006). For HER-2, immunostaining was scored according to the criteria specified in the HercepTest as follows: 0 = no staining, +1 faint, incomplete membranous staining, +2 moderate, complete membranous staining, +3 strong membranous staining of at least 10% of tumor cells. Cases interpreted as 0 or +1 were considered negative, whereas scores +2 or +3 were considered to indicate HER-2 overexpression. To determine Ki-67 scores, eight to 10 representative tumor areas (mostly at the periphery of the tumor) were selected and at least 1000 cells were counted. The number of positive cells per 1000 examined was expressed as a percentage as previously described (PENA et al., 1998; MILLANTA et al., 2002).

Statistical analysis. Data collected in the survey were statistically analyzed using the computer program STATISTICA (data analysis software system), StatSoft, Inc. (2006), Version 7.1. www.statsoft.com. To test the statistical significance of differences among samples parametric and nonparametric tests of significance were used. Statistical hypotheses were tested at the level of significance of P=0.05, i.e. the difference between samples was considered to be significant if P<0.05. The Kolmogorov-Smirnov test was used to check whether the data were normally distributed. Depending on the results of the Kolmogorov-Smirnov test, Single Factor analysis was performed using analysis of variance Kruskal-Wallis ANOVA. The Student-Newwman-Keuls (SNK) test was used as a complementary test to analyze the specific differences in Ki-67 expression among the HMG and in different tumor types.

Results

Dogs and tumors. The samples were from 146 dogs with a median age of 10 years (range: 4 to 15 years of age). The most common breeds affected were cross breeds (31), poodles (15), cocker spaniels (13), German shepherds (7), Yorkshire terriers (6), Pekingese (5) and four schnauzers, Irish setters and Siberian huskies. Other breeds were represented by three or fewer animals, and in 13 cases the breed was not specified.

Histologic examination yielded 110 (75.3%) malignant tumors (35 complex carcinomas, 28 tubulopapillary carcinomas, 17 solid carcinomas, 10 anaplastic carcinomas, 4 squamous cell carcinomas, 2 spindle cell carcinomas, 4 sarcomas, 2 carcinosarcomas, 8 carcinomas in benign tumors) and 36 (24.7%) benign tumors (14 complex adenomas, 8 simple adenomas, 9 benign mixed tumors, 1 fibroadenoma and 4 mammary hyperplasias).

The carcinomas studied included 50 (48.1%) well differentiated tumors (grade I), 35 (33.7%) moderately differentiated tumors (grade II) and 19 (18.3%) poorly differentiated tumors (grade III) (Table 1).

Immunohistochemistry. Immunohistochemistry for ERα, HER-2 and Ki-67 was performed on 51 malignant and 24 benign canine mammary lesions (Table 1 and 3).

ERα immunostaining was localized in the nuclei of normal, benign and malignant epithelial cells (Fig. 1.). Positive immunolabeling for ERα was detected in 18/75 (24%) mammary lesions, including 9/51 (17.6%) malignant tumors and 9/24 (37.5%) benign lesions (Table 2). The ERα expression was not significantly associated with tumor type or tumor grading.

Table 1. Histological malignancy grade of 104 canine mammary carcinomas

Histological type	HMG I	HMG II	HMG III	Total
Complex carcinoma	24	7	4	35
Simple (tubulopapillary) carcinoma	16	11	1	28
Simple (solid) carcinoma	1	10	6	17
Simple (anaplastic) carcinoma	-	4	6	10
Spindle cell carcinoma	-	2	-	2
Squamous cell carcinoma	1	1	2	4
Carcinoma in benign tumor	8	-	-	8
Total	50	35	19	104

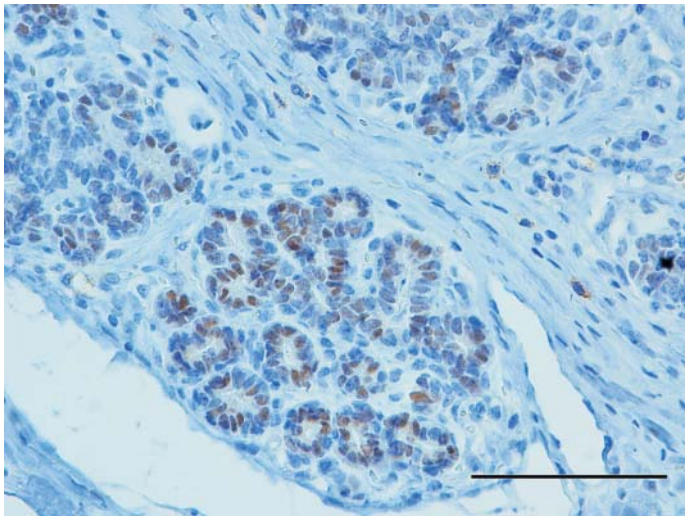

Fig. 1. Mammary gland, dog; moderate nuclear immunostaining of ERα in majority of acinar epithelial cells in mammary hyperplasia. IHC, scale bar 100 μm.

Positive HER-2 immunoreactivity was localized in the cell membranes of neoplastic cells (Fig. 2.), and positive staining in most cases was distributed uniformly throughout the neoplastic cell population, although the immunostaining intensities of cells of individual tumors showed variations. HER-2 expression was detected in 5/51 (9.8%) malignant tumors and none of the benign lesions (Table 2). HER-2 status was not significantly associated with histologic type or tumor grading.

Table 2. Expression of estrogen receptor α and HER-2 according to histologic classification

Tumor	ERα	HER2
Malignant (51)	9 (17.6%)	5 (9.8%)
Complex carcinoma	2	1
Simple (tubulopapillary) carcinoma	3	2
Simple (solid) carcinoma	1	0
Simple (anaplastic) carcinoma	0	1
Carcinoma in benign tumor	2	1
Squamous cell carcinoma	1	0
Benign (24)	9 (37.5%)	0 -
Complex adenoma	2	0
Simple adenoma	1	0
Benign mixed tumor	2	0
Adenosis	4	0
Total	18 (24%)	5 (9.8%)

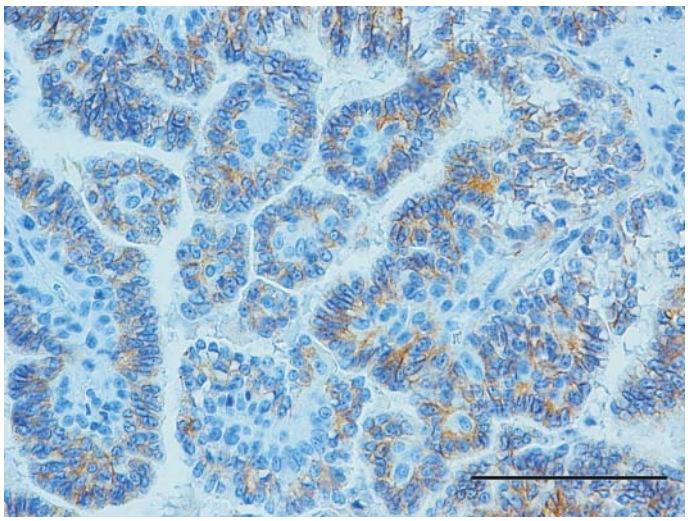

Fig. 2. Mammary gland, dog; moderate complete membranous staining (2+) of HER-2 in tubulopapillary carcinoma. IHC, scale bar 100 μm.

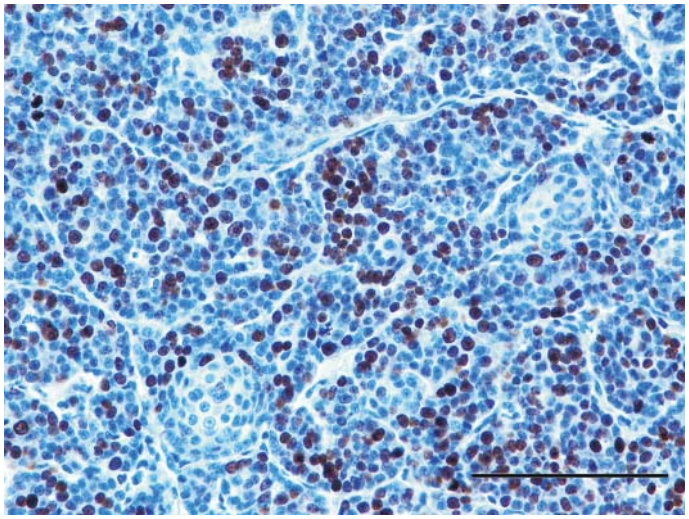

Fig. 3. Mammary gland, dog, strong nuclear immunostaining of epithelial cells (Ki-67 index 60%) in squamous cell carcinoma. IHC, scale bar 100 μm.

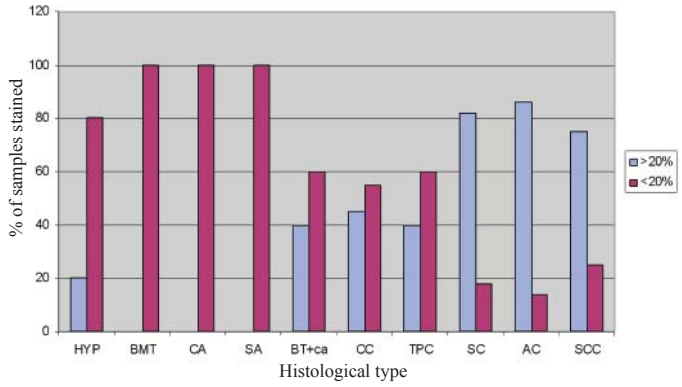

Fig. 4. Immunohistochemical expression of Ki-67 above and below 20% immunopositivity in canine mammary lesions (HYP-hyperplasias; BM-benign mammary tumor; CA-complex adenoma; SA-simple adenoma; BT+ca-carcinoma in benign tumor; CC-complex carcinoma; TPC-tubulopapillary carcinoma; SC-solid carcinoma; AC-anaplastic carcinoma; SCC-squamous cell carcinoma).

Table 3. Correlation between proliferative activity and some pathological variables in canine mammary tumors

	Ki-67 index			
	n	mean	SEM	P
Histology				
Benign	22	9.1	7.0	P<0.001
Malignant	48	29.5	19.7	
Histologic malignant grade				P = 0.002
HMG 1	16	16.3*	12.2	
HMG 2	18	33.4	20.9	
HMG 3	14	39.6	17.9	
Tumor type				P = 0.011
Complex carcinoma	11	27.6	16.7	
Tubulopapillary carcinoma	10	17.1	11.7	
Solid carcinoma	11	31.9	16.7	
Anaplastic carcinoma	7	46.6*	21.8	
Squamous cell carcinoma	4	44.5	28.8	
Carcinoma in benign tumor	5	17.7	14.2	

*SNK test separated HMG I from HMG II and HMG III (P<0.05) and tubulopapillary from anaplastic carcinomas (P<0.05)

Ki-67 (MIB-1) immunostaining had a diffuse staining pattern that was confined to the nuclei but had variable intensity of staining both within and between samples (Fig.3). Of the 75 tumors, 70 had positive staining, and five were discarded because they had no reaction to Ki-67. The majority of benign tumors had<20% immunopositive cells and the expression of Ki-67 was significantly higher in malignant lesions (P<0.001) (Table 3, Fig. 4.). Among the malignant tumors, the SNK test separated HMG I from HMG II and HMG III (P<0,05) but there was no significant difference in Ki-67 expression between HMG II and HMG III. In a comparison of the proliferative activity in different types of mammary carcinoma, anaplastic carcinomas had a significantly higher Ki-67 index than tubulopapillary carcinomas (P<0.05), whereas there were no significant differences among other types of carcinomas (Table 3).

Discussion

The frequencies of different types of mammary lesions and their distribution according to breed and age of female dogs in Croatia has not yet been established.

Considering the breed distribution, cross breeds, poodles and cocker spaniels were predominant, which is similar as in other studies (ZATLOUNKAL et al., 2005; HSU et al., 2009; MORRIS et al., 2009). The age at diagnosis ranged from 4 to 15 years, with a median of 10 years. This interval of risk age is in agreement with other studies (NIETO et al., 2000; SARLI et al., 2002; YANG et al., 2006; MORRIS et al., 2009).

An interesting finding in this study was the high percentage of malignant mammary tumors (75.3%). According to literature data, 41% to 53% of mammary tumors that occur in bitches are considered malignant (MISDORP et al., 1999; LANA et al., 2007), and only a few investigations have shown a higher percentage of malignant tumors in the investigated period (YANG et al., 2006; MORRIS et al., 2009). In the present study data considering tumor size and OH (ovariohysterectomy) status were not included, which might be helpful in explaining the high percentage of malignant tumors. It could be supposed that late stage tumor removal possibly resulted in such a high percentage of malignant mammary tumors, as well as the high number of intact females and spaying at an older age, although it is well known that later spaying does not reduce the risk for malignant tumors (LANA et al., 2007). However, in spite of this high percentage of malignant mammary tumors, according to WHO classification, the vast majority of malignant tumors were well differentiated adenocarcinomas, mostly complex and tubulopapillary, whereas special types of carcinomas and sarcomas were rare, which is similar as in other studies (RUNGSIPIPAT et al., 1999; MILLANTA et al., 2005; YANG et al., 2006; THUROCZY et al., 2007; HSU et al., 2009; MORRIS et al., 2009).

Another objective of this study was to investigate ERα, HER-2 and Ki-67 expression in selected dysplastic and neoplastic mammary lesions. Previous studies showed a decreasing percentage of ERα immunoreactivity with increasing malignancy (NIETO et al., 2000; MARTIN de las MULAS et al., 2005; YANG et al., 2006) and higher ERα expression in complex and mixed histologic subtypes of benign and malignant tumors (NIETO et al., 2000; MARTIN de las MULAS et al., 2005; MORRIS et al., 2009). Compared with other studies, our results showed a lower expression of ERα in benign (37.5%) and malignant (17.6%) canine mammary lesions. Some of the negative tumors did not contain additional normal or hyperplastic tissue and therefore it could not be determined if the loss of tissue antigenicity was due to fixation or processing problems. Similar results were described by NIETO et al. (2000).

In this study, HER-2 protein overexpression was observed in canine malignant mammary tumors, but not in benign lesions which is similar to the results of AHERN et al., (1996), MARTIN de las MULAS et al. (2003) and HSU et al. (2009). The percentage of HER-2

positive canine mammary carcinomas (9.8%) was lower than that reported in previous studies (19.1%, RUNGSIPIPAT et al., 1999; 17.6%, MARTIN de las MULAS et al., 2003; 21%, GAMA et al., 2008; 29.7%, HSU et al., 2009). HER-2 overexpression is related to a number of factors, including the sensitivity of the detection method, the level of gene expression or the stages of tumor samples (HSU et al., 2009). GOUVEA et al. (2006) compared the ability of different antibodies to detect HER-2 expression in human mammary carcinomas by IHC and found that results using monoclonal antibodies were more closely correlated with gene amplification assessed by fluorescent in situ hybridisation (FISH) than results using polyclonal antibodies, as was used in our study.

Unlike ERα and HER-2, Ki-67 showed good immunoreactivity in almost all investigated samples (70/75). As expected from previous studies (PENA et al., 1998; GERALDES et al., 2000; De MATOS et al., 2006; THUROCZY et al., 2007; MORRIS et al., 2009) Ki-67 showed increased percentages of positive nuclear staining with increasing histologic grade. We also identified a significantly higher Ki-67 index in anaplastic carcinomas compared to tubulopapillary carcinomas, in contrast with other studies, which did not find any correlation between Ki-67 index and histologic type. Due to the low number of samples with ERα and HER-2 expression, it was not possible to investigate the correlation between Ki-67, ERα and HER expression. However, immunohistochemical staining with Ki-67 antibody represents a robust marker of cell proliferation in routinely processed canine mammary tumors, and the Ki-67 index was related to tumor grade and tumor types.

Nowadays, despite the vast array of immunohistochemical studies of ERα, HER-2, Ki-67 and many other oncogenes and tumor suppressor genes in the literature (DE MATOS et al., 2006; GAMA at al., 2008; ZAIDAN DAGLI, 2008; MORRIS et al., 2009; FERREIRA et al., 2009), no such markers have been adopted for routine use by the veterinary profession (MORRIS, 2010). Also, many different scoring systems are applied for evaluation of immunohistochemical data (PENA et al., 1998; MARTIN de las MULAS et al., 2005; MILLANTA et al., 2005; MORRIS et al., 2009) which partly results in a wide range of results. However, even small immunohistochemical studies may contribute to finding prognostic markers, that will eventually improve diagnosis and treatment of canine mammary tumors.

Acknowledgements

The work was supported by Ministry of Science, Education and Sport grant No. 053-0532264-2260.

References

AHERN, T. E., R. C. BIRD, A. E. CHURCH BIRD, L. G. WOLFE (1996): Expression of the oncogene c-*erb*B-2 in canine mammary cancers and tumor-derived cell lines. Am. J. Vet. Res. 57, 693-696.

BRATULIĆ, M., Ž. GRABAREVIĆ, B. ARTUKOVIĆ, D. CAPAK (1996): Number of nucleoli and nucleolar organizer regions per nucleus and nucleolus - prognostic value in canine mammary tumors. Vet. Pathol. 33, 527-532.

DE MATOS, A. J. F., C. C. C. LOPES, A. M. FAUSTINO, J. G. V. CALVALHERA, M. S. A. DOS SANTOS, G. R. RUTTEMAN, M. D. R. M. GARTNER (2006): MIB-1 labeling indices according to clinico-pathological variables in canine mammary tumours: a multivariate study. Anticancer Res. 26, 1821-1826.

FERREIRA, E., A. C. BERTAGNOLLI, M. F. CAVALCANTI, F. C. SCHMITT, G. D. CASSALI (2009): The relationship between tumour size and expression of prognostic markers in benign and malignant canine mammary tumours. Vet. Comp. Oncol. 7, 230-235.

GAMA, A., A. ALVES, F. SCHMITT (2008): Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: application of the human classification. Virchows Arch. 453, 123-132.

GERALDES, M., F. GÄRTNER, F. SCHMITT (2000): Immunohistochemical study of hormonal receptors and cell proliferation in normal canine mammary glands and spontaneous mammary tumors. Vet. Rec. 146, 403-406.

GERDES, J., H. LEMKE, H. BAISCH, H. H. WACKER, U. SCHWAB, H. STEIN (1984): Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J. Immunol. 133, 1710-1715.

GINN, P. E., J. E. K. L. MANSELL, P. M. RAKICK (2007): Skin and appendages. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals. (Maxie, E.G., Ed.). Elsevier, Saunders. Fifth edition. pp. 553-781.

GOUVEA, A. P., F. MILANEZI, S. J. OLSON, D. LEITAO, F. C. SCHMITT, H. GOBBI (2006): Selecting antibodies to detect HER2 overxpression by immunohistochemistry in invasive mammary carcinomas. Appl. Immunohistochem. Mol. Morphol. 14, 103-108.

HSU, W. L., H. M. HUANG, J. W. LIAO, M. L. WONG, S. C. CHANG (2009): Increased survival in dogs with malignant mammary tumours overexpressing HER-2 protein and detection of a silent single nucleotide polymorphism in the canine HER-2 gene. Vet. J. 180, 116-123.

KILLEEN, A. A. (2004): Principles of molecular pathology. Humana Press Inc. Totowa, New Jersey. pp. 185-209.

LANA, S. E., G. R. RUTTEMAN, S. J. WITHROW (2007): Tumors of the mammary gland. In: Small Animal Clinical Oncology. (Withrow, S. J., D. M. Vail, Eds.). Saunders, Elsevier. St. Louis, Missouri. pp. 619-636.

LESTER, S. (2010): The Breast. In: Robbins and Cotran Pathologic Basis of Disease. (Kumar, V., A. B. Abbas, N. Fausto, J. C. Aster, Eds.). 8th ed., Saunders, Elsevier. Philadelphia. pp. 1065-1095.

MARTIN de las MULAS, J., J. ORDÁS, Y. MILLÁN, V. FERNÁNDEZ-SORIA, S. RAMÓN Y CAJAL (2003): Oncogene HER-2 in canine mammary gland carcinomas: an immunohistochemical and chromagenic in situ hybridization study. Breast Cancer Res. Treat. 80, 363-367.

MARTIN de las MULAS, J., Y. MILLÁN, R. DIOS (2005): A prospective analysis of immunohistochemically determined estrogen receptor α and progesterone receptor expression and host and tumor factors as predictors of disease-free period in mammary tumors of the dog. Vet. Pathol. 42, 200-212.

MILLANTA, F., G. LAZZERI, M. MAZZEI, I. VANNOZZI, A. POLI (2002): MIB-1 labeling index in feline dysplastic and neoplastic mammary lesions and its relationship with postsurgical prognosis. Vet. Pathol. 39, 120-126.

MILLANTA, F., M. CALANDRELLA, G. BARI, M. NICCOLINI, I. VANNAOZZI, A. POLI (2005): Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. Res. Vet. Sci. 79, 225-232.

MISDORP, W., R. W. ELSE, E. HELLMEN, T. P. LIPSCOMB (1999): WHO Histological Classification of Tumors of Domestic Animals. Histological Classification of Mammary Tumors of the Dog and the Cat. 2nd series, Volume VII. Washington DC: Armed Forces Institute of Pathology.

MISDORP, W. (2002): Tumors of the mammary gland. In: Tumors in Domestic Animals. (Meuten, D. J., Ed.). Iowa State Press. Ames. pp. 575-606.

MORRIS, J. S., C. NIXON, O. J. A. KING, I. M. MORGAN, A. W. PHILBEY (2009): Expression of TopBP1 in canine mammary neoplasia in relation to histological type, Ki67, ERα and p53. Vet. J. 179, 422-429.

MORRIS, J. S. (2010): Improving the diagnosis and treatment of canine mammary tumours: Immunohistochemical markers as a prognostic tools. Vet. J. 184, 3-4.

MUTO, T., S. WAKUI, H. TAKAHASHI, S. MAEKAWA, T. MASAOKA, S. USHIGOME, M. FURUSATO (2000): p53 gene mutations occuring in spontaneous benign and malignant mammary tumors of the dog. Vet. Pathol. 37, 248-253.

NIETO, A., L. PENA, M. D. PEREZ-ALENZA, M. A. SANCHEZ, J. M. FLORES, M. CASTANO (2000): Immunohistologic detection of estrogen receptor alpha in canine mammary tumors: clinical and pathologic associations and prognostic significance. Vet. Pathol. 37, 239-247.

PENA, L., A. I. NIETO, D. PEREZALENZA, P. CUESTA, M. CASTANO (1998): Immunohistochemical detection of Ki-67 and PCNA in canine mammary tumors - relationship to clinical and pathologic variables. J. Vet. Diagn. Invest. 3, 237-246.

PEREZ ALENZA, M. D., G. R. RUTTEMAN, N. J. KUIPERS-DIJKSHOORN, L. PENA, A. MONTOYA (1995): DNA flow cytometry of canine mammary tumours: the relationship of DNA ploidy and S-phase fraction to clinical and histological features. Res. Vet. Sci. 58, 238-243.

PEREZ ALENZA, M. D., L. PENA, N. del CASTILLO, A. I. NIETO (2000): Factors influencing the incidence and prognosis of canine mammary tumours. J. Small Anim. Pract. 41, 287-291.

ROSS, J. S., J. A. FLETCHER (1999): The HER-2 neu oncogene: prognostic factor, predictive factor and target for therapy. Semin. Cancer Biol. 9, 125-138.

RUNGSIPIPAT, A., S. TATEYAMA, R. YAMAGUCHI, K. UCHIDA, N. MIYOSHI, T. HAYASHI (1999): Immunohistochemical analysis of c-*yes* and c-*erb*B-2 oncogene products and p53 tumor suppressor protein in canine mammary tumors. J. Vet. Med. Sci. 61, 27-32.

SAHIN, A. A. (2000): Biologic and clinical significance of HER-2/neu (cerB-2) in breast cancer. Adv. Anat. Pathol. 7, 158-166.

SARLI, G., R. PREZIOSI, C. BENAZZI, G. CASTELLANI, P. S. MARCATO (2002): Prognostic value of histologic stage and proliferative activity in canine malignant mammary tumors. J. Vet. Diagn. Invest. 14, 25-34.

SORENMO, K. U., F. S. SHOFER, M. H. GOLDSCHMIDT (2000): Effect of spaying and timing of spaying on survival of dogs with mammary carcinoma. J. Vet. Intern. Med. 14, 266-270.

THUROCZY, J., G. J. K. REISVAAG, E. PERGE, A. TIBOLD, J. SZILAGYI, L. BALOGH (2007): Immunohistochemical detection of progesterone and cellular proliferation in canine mammary tumours. J. Comp. Pathol. 137, 122-129.

YANG, W. Y., C. H. LIU, C. J. CHANG, C. C. LEE, K. J. CHANG, C. T. LIN (2006): Proliferative activity, apoptosis and expression of oestrogen receptor and Bcl-2 oncoprotein in canine mammary tumours. J. Comp. Pathol. 134, 70-79.

ZAIDAN DAGLI, M. L. (2008): The search for suitable prognostic markers for canine mammary tumors: a promising outlook. Vet. J. 177, 3-5.

ZATLOUKAL, J., J. LORENZOVA, F. TICHY, A. NEČAS, H. KECOVA, P. KOHOUT (2005): Breed and age as a risk factors for canine mammary tumours. Acta Vet. Brno. 74, 103-109.

Received: 29 October 2010
Accepted: 17 March 2011

GUDAN KURILJ, A., M. HOHŠTETER, B. ARTUKOVIĆ, K. SEVERIN, I.-C. ŠOŠTARIĆ-ZUCKERMANN, A. BECK, S. SEIWERTH, R. SABOČANEC, Ž. GRABAREVIĆ: Histopatološko vrednovanje i imunohistokemijska analiza estrogenskih receptora α, HER-2 i Ki-67 u displastičnom i neoplastičnom tkivu mliječne žlijezde kuja. Vet. arhiv 81, 709-722, 2011.

SAŽETAK

U radu je prikazana učestalost različitih tipova lezija mliječne žlijezde u kuja te stupanj malignosti tumora. Sto četrdeset i šest lezija mliječne žlijezde je klasificirano prema kriterijima Svjetske zdravstvene organizacije. Odabrane lezije (51 maligna i 24 benigne) bile su podvrgnute imunohistokemijskoj analizi radi utvrđivanja ekspresije estrogenskih receptora alfa (ERα), HER-2 i Ki-67 te njihovoga značenja u odnosu na neke karakteristike tumora. Tumori mliječne žlijezde najčešće su bili utvrđeni u križane pasmine, pudla, koker španijela i njemačkih ovčara, a dijagnosticirani su prosječno u dobi od 10 godina (raspon od 4 do 15 godina). Klasifikacija svih lezija mliječne žlijezde kuja pokazala je 75,3% malignih i 24,7% benignih tumora. Ekspresija ERα bila je utvrđena u 9/24 (37,5%) benignih i 9/51 (17,6%) malignih tumora. Ekspresija HER-2 proteina utvrđena je u 5/51 (9,8%) malignih tumora, a u benignih tumora nije bila utvrđena. Ki-67 indeks bio je veći u malignih (aritmetička sredina 29,5) nego u benignih (aritmetička sredina 9,1) tumora ($P<0,001$) te značajno veći u anaplastičnih nego u tubulopapilarnih karcinoma ($P<0,05$).

Ključne riječi: tumori, mliječna žlijezda, kuja, ERα, HER-2, Ki-67