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The number of mitoses in simple and complex type carcinomas of the mammary gland in dogs

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According to the WHO classification mammary gland tumours in dogs are classified into simple and complex type. General opinion is that the complex type carcinomas have better prognosis than the simple type carcinomas. The aim of this research was to quantify and compare the mitotic activity in both types of carcinomas. Sixty-five simple type and 99 complex type carcinomas were included in the study and two methods for counting of mitoses were compared: mitotic activity index (MAI) and number of mitoses per square millimetre (M/mm^2). Both methods showed significant ($P=0.0001$ for MAI; and $P=0.0089$ for M/mm^2) differences between simple and complex type carcinomas with respect to the number of mitoses. The number of mitoses in individual tumours varied from 0 to 95 per ten high power fields in simple type carcinomas and from 0 to 57 in complex type carcinomas. The average number of mitoses was higher when only one tumour was present and lower when there were multiple tumours. Our results confirm the lower mitotic potential of the epithelial parts of complex type mammary gland carcinomas in comparison with simple type carcinomas. This fact certainly contributes to better prognosis of the disease, especially if myoepithelial cells are a predominant component of the tumour.

Key words: mammary neoplasms; dogs; mitotic index

Introduction

Mammary gland tumours are the most common neoplasias in dogs. They represent 25% to 50% of all tumours encountered in this species. WHO classification of animal tumours divides mammary gland tumours (benign and malignant) into simple and complex type. The simple type tumours consist of epithelial or myoepithelial cells only, whereas the complex type tumours consist of both types of cells in different proportions. Most authors agree that the complex type carcinomas have better prognosis and that the epithelial component of tumour is a fraction generally responsible for the metastatic spreading of tumour cells to regional and distant lymph nodes and other target locations.[1,2]

High mitotic activity is a feature characteristic for highly aggressive tumours. The num-

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ber of mitoses has been established as an important prognostic factor in breast cancer in women. It serves as one of the indicators of the tumour proliferation rate, although the techniques used for counting of mitoses are rather inconsistent.[3-7] In dogs, a relationship between the number of mitoses and the prognosis of mammary gland tumours is not clearly defined. The aim of this study was to evaluate the number of mitoses in two types of mammary gland tumours in dogs:

a) the simple type carcinomas consisting of epithelial cells only, and

b) the epithelial parts of the complex type carcinomas.

Materials and methods

Mitoses were counted in routinely prepared, paraffin embedded and haematoxylin eosin stained 4mm thick tissue sections, using a light microscope (high power field – 450mm field diameter, NA 0.70, magnification 400x) and image analysis system Prodit 5.2 (Buro Medische Automatisering – BMA, The Netherlands). Mitoses in 10 high power fields were counted according to the rules recommended by van Diest and coworkers which were followed as much as possible.[8] Some minor modifications were made due to the differences in architecture of human and animal mammary gland tumours. Necrotic, inflamed or haemorrhagic parts of tumours were avoided and so were the cartilaginous and bony parts. In the complex type carcinomas, only the tissue with epithelial cells was analysed for mitotic activity, whereas the parts consisting entirely of myoepithelial cells were excluded from the analysis. Only the cells with clear signs of mitosis were counted, others were ignored. The number of mitoses assessed as mitotic activity index (MAI) was correlated to the number of mitoses calculated per square millimetre (M/mm^2). The mitosis mean value, standard error (±SE), maximum number and mediana were calculated for each group of tumours examined by the two methods. Differences between the methods and tumour groups were evaluated statistically by the analysis of variance, Student's t-test, Pearson's correlation coefficients and Mann-Whitney (Wilcoxon) test, using statistics package Statgraphics.[9]

Sixty-five simple type and 99 complex type mammary gland carcinomas were analysed. As mammary gland tumours in dogs may appear as single (solitary) tumours, invading only one mammary gland or as multiple tumours invading two or more mammary glands, we divided both main groups of carcinomas (simple and complex type) into subgroups according to the number of primary tumours diagnosed. Using this classification, 29 simple type carcinomas were categorised as single and 36 as multiple. Among the complex type carcinomas, 51 were single and 48 multiple. Depending on the quantity of myoepithelial cells, the complex type carcinomas were further divided into two subgroups: a) tumours consisting predominantly of epithelial cells (74 tumours) and b) tumours consisting predominantly of myoepithelial cells (27 tumours). In this last group, 10 tumours were solitary and 17 were multiple.

Results

Results are summarized in Tables 1 and 2. Distribution plot of mitoses in individual groups is presented in Figure 1.

Simple type carcinomas and epithelial parts of the complex type carcinomas differred significantly in the number of mitoses ($P=0.0001$, for the MAI method; $P=0.0089$, for the M/mm^2 method). Values obtained by the two methods were highly correlated ($r=1.000$, $P=0.0001$). The number of mitoses was considerably higher in solid tumours, classified as simple type carcinomas or complex type

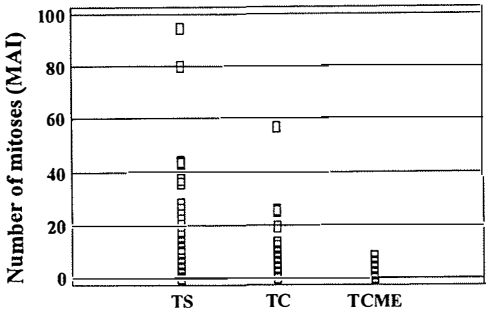

Figure 1. Scatterplot of the number of mitoses calculated as mitotic activity index (MAI) in simple (TS) and complex type (TC) mammary gland carcinoma and in complex type carcinoma with the prevalence of myoepithelial cells (TCME).

Table 1. The mean number of mitoses (SE ±)in simple and complex type carcinomas and complex type carcinomas with the prevalence of myoepithelial cells (ME), calculated per ten high power fields (MAI) and as the mean number per square millimetre (M/mm^2)

Tumour type	MAI	M/mm^2
simple	12.02	7.69
n = 65	SE ± 1.79	SE ± 1.40
complex	4.13	0.86
n = 99	SE ± 0.72	SE ± 0.57
complex – ME	2.03	1.33
n = 27	SE ± 0.23	SE ± 0.14

carcinomas with solid parts. MAI varied from 0 to 95 per ten high power field in simple type carcinomas, from 0 to 57 in complex type carcinomas and from 0 to 8 in predominantly myoepithelial complex type carcinomas. Significant difference was also found between the subgroups of solitary and multiple tumours in simple type carcinomas (P=0.0361).

Discussion

A limited number of published papers examine and discuss the importance of counting of mitoses in the mammary gland tumours in dogs. In most of those papers, mitoses are mentioned only briefly and their significance is not fully analysed and evaluated with respect to the prognosis of the disease.[10] The reason that counting of mitoses in mammary gland tumours in dogs has not been widely accepted as a prognostic tool may be associated with morphological specificities of the mammary gland tumours in this species. Mammary gland tumours in dogs often contain a large number of myoepithelial cells, which are seldom found in breast tumours in

Table 2. The mean, standard error (SE ±), maximum number and mediana for the number of mitoses assessed as MAI in solitary and multiple tumours of simple and complex type and in complex type tumours with the prevalence of myoepithelial cells (ME)

Tumour type	MAI	SE ±	Maximal	Mediana
Simple				
solitary	16.83	2.29	95	6.0
multiple	8.14	1.19	44	3.0
Complex				
solitary	4.75	0.88	57	2.0
multiple	3.48	0.49	26	2.0
Complex-ME				
solitary – ME	1.90	0.20	5	1.5
multiple – ME	2.29	0.24	8	2.0

women. In dogs, this type of cells is present in about 50% of tumours, but is less common in malignant tumours.[1,11] Hampe and Misdorp[1] describe simple type carcinomas in bitches as highly infiltrative tumours that readily produce metastases to regional and distant lymph nodes and lungs. Survival period after diagnosis and surgery of this type of carcinomas is usually short. On the other hand, the complex type carcinomas grow in a more expansive way and metastases are not so common. Malignant part of the complex type carcinomas that expresses its metastatic potential is usually the epithelial component of the tumour. Survival period of animals with the complex type carcinomas is relatively long. Expansive, nodular and lobulated growth is common in complex type carcinomas, whereas growth along the lymph vessels is rare. Histological distinction between highly differentiated carcinomas of this type and complex adenomas can be very difficult. Numerous mitoses, high cellularity and relatively large amount of necrosis are usual indicators of malignancy. These criteria, however, are rather subjective, therefore, the number of mitoses could serve as a decisive criterion in dubious cases.

Lack of standardisation of methods used for counting the mitoses in mammary gland tumours is not the only problem encountered in diagnosis and prognosis of animal tumours. In dogs, nearly half of the diagnosed mammary gland tumours are multicentric or multiple.[12] They are not necessarily all malignant and often they are of different cellular origin. Malignant neoplasms may occur together with benign tumours or another malignant tumour of completely different type.[13,14] A pathologist often gets only one tumour or just a part of it for histopathological examination and that may not necessarily be the part responsible for metastases or recurrence of the tumour.

The objectives of our research were to quantify and compare the mitotic activity in simple and complex type carcinomas (including complex type carcinomas with the prevalence of myoepithelial cells) in order to evaluate the importance of counting the mitoses for the prognosis of mammary gland tumours in dogs. Furthermore, we wanted to contribute to the efforts to standardise the methods used for counting the mitoses in mammary gland tumours. We also evaluated the number of mitoses in solitary and multiple tumours, expecting that these two groups would not differ significantly.

Both methods (MAI and M/mm^2) indicate that the simple type carcinomas and the epithelial parts of the complex type carcinomas differ significantly in the number of mitoses (P=0.0001 for MAI, and P=0.0089 for and M/mm^2). As we expected, the number of mitoses was higher in solid carcinomas, regardless whether they were classified as simple type carcinomas or complex type carcinomas with solid parts. The number of mitoses in epithelial parts of the complex type mammary gland carcinomas was significantly lower than in the epithelial simple type carcinomas. These results disagree with some previously published observations. Misdorp and Hart[13] found no differences between the complex and simple type carcinomas with respect to the number of mitoses. They concluded that neither mitoses nor other constituents of histological grade (differentiation, anaplasia) could be used for the prognosis of disease. However, they assumed that the complex type carcinomas have better prognosis than simple type carcinomas, due to the lower grade of anaplasia. In contrast to some other opinions, [15,16] Bostock[17] suggested that histological characteristics, including mitoses, are less important for the prognosis of canine and feline mammary gland tumours than the mode of growth at the edge of the tumour mass.

We found no significant differences in the number of mitoses between the carcinomas of complex type belonging to the subgroups

of solitary and multiple tumours, regardless the quantity of myoepithelial cells. However, in simple type carcinomas, differences in mitotic activity between the subgroups of solitary and multiple tumours were found. This findings were somewhat unexpected. They may be associated with different mode of development of solitary and multiple tumours. In case of multiple tumours, initial hormonal imbalances that affect several mammary glands, may lead to the development of preneoplastic lesions, which may gradually transform into the lower grade neoplastic lesions with lower number of mitoses and slower rate of growth. On the contrary, solitary tumours often exhibit faster mode of development and growth that is reflected also in higher number of mitoses.

Conclusions

The number of mitoses could be an important prognostic factor in the mammary gland tumours in dogs; a better prognosis can be predicted for the complex type carcinomas than for the simple type; more mitoses and more rapid growth can be expected in solitary tumours than in multiple tumours. The worst prognosis may be expected in solid solitary carcinomas and the best one when tumours are multiple and of the complex type, especially in tumours with the prevalence of myoepithelial cells. The number of mitoses could be a decisive criterion in cases where the distinction between the benign and malignant tumours is not clear and morphological evaluation alone is not conclusive. For the final evaluation of the number of mitoses as a prognostic factor in mammary gland tumours in dogs, further studies, employing a larger number of samples and a long-term follow up of the patients, are necessary.

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