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PAPER

Conventional and Doppler ultrasound for the differentiation of benign and malignant canine mammary tumours

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Objectives: The aim of this study was to evaluate the sensitivity of conventional and Doppler ultrasound for differentiation of benign and malignant mammary tumours in female dogs.

Methods: Mammary tumours were evaluated from 60 animals and divided into two distinct groups, group 1 (benign tumours) and group 2 (malignant tumours). The tumours were assessed by conventional ultrasound, Doppler ultrasound mode, histopathology and immunohistochemical detection of vascular endothelial growth factor.

Results: Conventional ultrasound examination was found to be ineffective in separating tumours into the two experimental groups. Similarly, using colour-flow Doppler ultrasound, no correlation was found between the presence of vascularisation and its characteristics between the two groups. Triplex Doppler ultrasound yielded average maximum velocities of 28·71 cm/s for malignant and 19·91 cm/s for benign tumours, which were significantly different (P=0·01). For vascular endothelial growth factor, an average score of 2·22 was found for group 2 and 1·66 for group 1 (P=0·03). Positive correlations were found between vascular endothelial growth factor and presence of vascularisation (P=0·04 and r=0·3658) and between vascular endothelial growth factor and maximum velocity (P=0·03 and r=0·3913).

Clinical Significance: Doppler evaluation may be used to predict malignancy of mammary tumours in bitches.

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INTRODUCTION

Canine mammary tumours are one of the most common neoplastic disorders in veterinary medicine and have been widely investigated. Moreover, canine mammary tumours may act as a possible model for the study of breast cancer in women (Martins and Ferreira 2003). Canine mammary neoplasms constitute approximately 52% of all tumours that affect bitches. Approximately 50% of the mammary masses are malignant (Queiroga and Lopes 2002).

Complete surgical resection with wide margins is the treatment of choice, especially when there is no evidence of metastasis, except for patients with inflammatory carcinomas. Chemotherapy and radiotherapy have low antitumoural activity against mammary cancer in companion animals (Lana and others 2007).

Ultrasonography has been used to assess the tissue composition and vascularity of canine mammary tumours (Nyman and others 2006a). However, the routine use of ultrasound for assessment of mammary neoplasms in female dogs has not been widely performed and there are few studies correlating gross and microscopic findings with the results of ultrasound imaging.

Some conventional ultrasound findings may aid in establishing tumour type. The heterogeneity of tumour tissue, irregular surface contour, the presence of unclear margins, posterior acoustic attenuation, skin thickening (hyperechogenicity) and the presence of calcifications may indicate malignancy (Paulinelli and others 2002, Lucena 2006). Regular margins are suggestive of benign lesions. By contrast, malignant tumours are more likely to present with irregular margins and acoustic shadowing (Bulnes and others 1998; Calas and others 2007).

A possible limitation of mammary ultrasonography in veterinary medicine is the inability to detect small lesions or microcalcifications that may lead to false negative results (Lana and others 2007). However, ultrasound is widely used for detection of abdominal and lymph node (axillary and superficial inguinal) metastasis (Zuki and Boyd 2004).

Doppler mode ultrasound is a relatively recent diagnostic tool in veterinary medicine. In association with conventional ultrasound imaging, Doppler mode ultrasound can give real-time information about vascularisation and haemodynamic aspects of blood vessel flow (Carvalho and others 2008). Doppler ultrasonography allows the measurement of maximum velocity (systole), minimum velocity (diastole), quantification of vessel density, systole/diastole ratio, mean velocity and resistance index (RI) and pulsatility index (PI) (Martins and others 2002).

Changes in tumour blood flow, such as increases in peak systolic velocity, may be associated with malignancy in humans. The presence of turbulent flow, high-impedance pulsatile signal (penetration), increased maximum velocity and venous flow are also indicative of malignant lesions (Dock and others 1991, Lucena 2006).

Angiogenesis is a complex process, characterised by neovascularisation from a pre-existing vascular network, and is essential for tumour growth and metastasis (Restucci and others 2002). Tumours can induce vascular neoformation and increase the size of vessels through a combination of tumour- and tissue-derived pro- and antiangiogenic factors. (Lucena 2006). Angiogenic factors including endothelial growth factor, angiogenin and alpha V beta 3 integrin ($\alpha_V\beta_3$) stimulate the formation of blood vessels. In contrast, antiangiogenic factors such as metalloproteinase inhibitors and vascular growth factor antagonists (angiogenin and $\alpha_V\beta_3$ integrin antagonists) impair neovascularisation.

Vascular endothelial growth factor (VEGF) is the most commonly investigated pro-angiogenic factor in current human oncology (Rebêlo 2009). However, there are few studies focused on VEGF in veterinary oncology, and there are no reports correlating this factor to tumoural haemodynamics (Restucci and others 2002, Kato and others 2007, Qiu and others 2008, Al Dissi and others 2010).

There are no reports on the use of conventional ultrasonography and Doppler Triplex mode for the assessment and differentiation of benign and malignant mammary neoplasms in bitches, as reported in human patients. Therefore, the aims of the current study were to describe the ultrasound findings regarding mammary tumours in bitches and to evaluate the effectiveness of Doppler Triplex mode on the differentiation of mammary neoplasms.

MATERIALS AND METHODS

Animals

This study was performed in accordance with the UK Veterinary Surgeons Act for Ethics and Welfare in animal experimentation. Sixty female dogs from various breeds, aged between two and eight-years old, were evaluated.

Anamnesis, general and specific physical examination (localisation, size, adherence, regularity of the surface tumour and presence of ulceration), regional lymph node palpation and radiographic surveys were performed in all animals as inclusion criteria for the study. Radiographs were performed to detect the presence of pulmonary metastases and assess the cardiac silhouette.

The animals in the current study had no physical or radiographic abnormalities (areas resembling pulmonary metastasis) that would preclude surgical treatment.

Experimental design

Using a prospective study design, 60 female dogs with mammary neoplasms were recruited. After ultrasound examination of mammary masses and histopathological diagnosis of the type of neoplasm, animals were divided into two experimental groups: dogs with benign tumours (group 1) and dogs with malignant tumours (group 2) (Misdrop and others 1999).

Diagnostic imaging

The ultrasonography was performed by a single evaluator experienced in ultrasonographic examinations. The ultrasound examination was performed prior to histological identification of tumour type according to Misdrop and others (1999).

Ultrasound was performed with a 12·0 MHz linear transducer using MyLab VET 30 ultrasound equipment (ESAOTE, Sao Paulo, Brazil). Echotexture of the parenchyma, margin regularity and presence of other ultrasonographic findings in the mammary masses were noted. Regional lymph nodes and the whole abdominal cavity were evaluated in order to exclude the presence of metastasis. The patients were not sedated during the examinations.

Colour-flow Doppler enabled visualisation of the presence and type of blood flow (laminar or turbulent) in mammary tumours. Triplex Doppler was used to assess the maximum velocity (peak systolic velocity), minimum velocity, RI and PI of neoplasms.

The whole tumour mass was assessed in two dominant planes (transverse and longitudinal) using B mode and Doppler Triplex mode. Pulsed Doppler was activated for the determination of sample volume by uniform insonation method. The cursor was positioned in an area of the vessel with apertures to measure the spectral trace of flow spectral curve and vascular index obtained automatically following software identification of the ultrasonic scanner for each waveform.

The power Doppler function was employed to increase the sensitivity of the blood flow measurements and to transform the examination from angle-independent to insonation or incident angle (Drost 2007).

Histopathological and immunohistochemical (VEGF) diagnosis

Samples of the mammary tumours were collected after mastectomy for histopathological evaluation. Samples containing tumoural and healthy tissue as assessed macroscopically were submitted. For small tumours (<1 cm), no ultrasound orientation was performed for tissue sampling. The sampling for histopathological examination of larger tumours was ultrasound-guided. The ultrasound images stored prior to mastectomy were consulted to optimise the tissue sampling.

The macroscopic appearance of each neoplasm was evaluated. Multiple fragments were fixed in 10% formaldehyde solution buffered with phosphates to a pH of 7·4, routinely processed and embedded in paraffin. Sections of 5 µm were obtained using a microtome. The slides were stained with haematoxylin and eosin. Optical microscopy was used to classify the neoplasms histologically as benign or malignant according to the criteria recommended by the World Health Organization (Misdrop and others 1999). The samples were assessed by the Department of Veterinary Pathology of the University of Brasília.

Immunohistochemistry was performed using a polymerase kit (NovoLink TM Polymer detection system) and a VEGF-specific antibody (Mouse monoclonal VG-1-VEGF abcam) to evaluate the expression of tumoural antigens (Turley and others 1998, Rebêlo 2009). Immunoreactions were analysed semi-quantitatively by a single examiner, who was blind to the type of mammary neoplasm, according to the intensity of the brownish colour of the neoplastic cell cytoplasm using the following scores: 1=absence of colour; 2=weak colour; 3=medium colour and 4=strong colour (Turley and others 1998). VEGF values were correlated to the variables assessed by Doppler ultrasound (presence or absence of vascularisation).

Statistical analysis

Data were previously tested for normality of residuals and homogeneity of variances (F test).

Raw and transformed averages were evaluated by analysis of variance. In addition, the t-test for paired samples was applied for all vascular indexes. For significant values, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Nonparametric data (echotexture, margin regularity, other ultrasonographic findings and presence of vascularisation) were analysed using Fischer's exact test for the analysis of the conventional ultrasound. Pearson's chi-square test was performed for the measurement of VEGF and flow velocity vascularisation. Spearman's rank correlation test was used for correlation of VEGF and presence of vascularisation.

RESULTS

Histopathologic diagnosis

After mastectomy and histopathologic diagnosis, 24 tumours were identified as benign (40%) and 36 as malignant (60%). Each dog had only one tumour. The benign group (group 1) was composed of mammary adenomas (n=8), fibroadenomas (n=5), mixed benign tumours (n=11). The malignant group (group 2) was composed of simple tubular carcinomas (9), complex tubular carcinomas (13), complex tubulopapilliferous carcinomas (4), fusiform cell tumours (1), simple solid carcinomas (6) and complex solid carcinomas (3).

Conventional ultrasound

Using conventional ultrasound examination, 22 benign mammary tumours showed regular margins and 2 showed irregular margins. Of the malignant tumours, 27 showed regular margins and 9 showed irregular margins. (Fig 1). When margin regularity was compared between the malignant and benign groups, no difference was found (P=0·17).

With respect to echotexture of tumoural parenchyma, 18 benign tumours and 16 malignant tumours showed homogeneous parenchymal echotexture, while 6 benign and 20 malignant tumours showed heterogeneous parenchymal echotexture (Fig 1). No difference was found between groups 1 and 2 (P=0·28).

In mammary tumours showing heterogeneous echotexture, cystic area (1 malignant tumour), hyperechogenic foci (6 benign, 10 malignant), cystic areas with hyperechogenic foci (2 benign, 7 malignant) and presence of acoustic shadowing (1 malignant) were seen. No difference was found between the two groups (P=0·51; Table 1).

Doppler mode

Using colour-flow Doppler, 13 benign and 16 malignant neoplasms showed no detectable vascularisation. Vascularisation was observed in 11 benign tumours and 20 malignant tumours (Fig 2). No difference was found between the two groups (P=0·75). Likewise, no difference was seen in the type of vascularisation between the two groups (P=0·70).

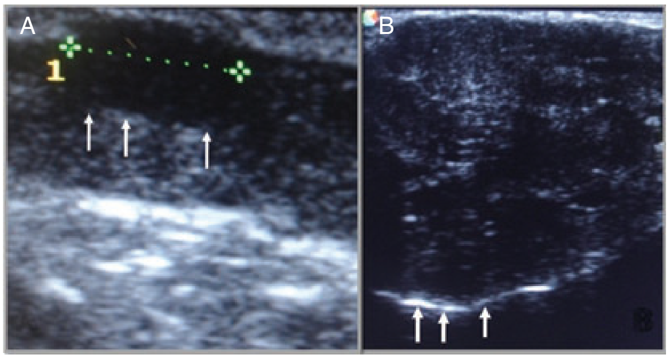

FIG 1. Sonographic image of a mammary neoplasm in a female dog. Note the surface regularity (A) and surface irregularity (B) of the tumour (arrows). Note homogeneous (A) and heterogeneous (B) tumoural parenchyma

Table 1. Ultrasound findings for mammary neoplasms in female dogs according to experimental groups

Variables	Frequency: Benign tumours	Frequency: Malignant tumours	P value
Other findings			
Homogeneous	18	16	
Cystic areas	0	1	0·51
Hyperechogenic foci	6	10	
Cysts and hyperechogenic foci	2	7	
Acoustic shadowing clean	0	1	

Contingency table for chi-square test at 5% of significance level

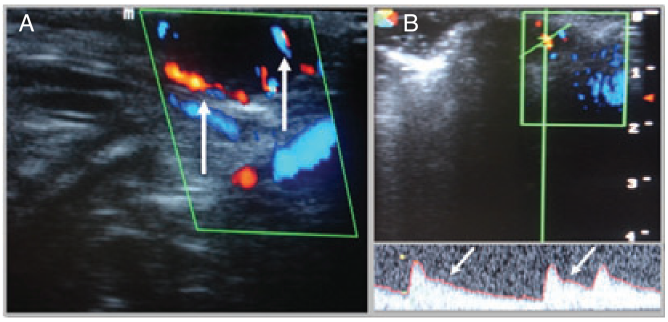

FIG 2. Sonographic image with colour-flow Doppler of a malignant mammary tumour in a female dog. (A) Note the presence of tumoural neovascularisation (arrows). (B) Waveforms of tumoural neovascularisation (arrow)

Table 2. Averages of vascular indices using Doppler ultrasound for mammary neoplasms in female dogs according to experimental groups

Variables	Benign tumours	Malignant tumours	P value
Maximum velocity	19·19 ±4·72	28·71 ±6·83	0·01*
Minimum velocity	7·17 ±1·65	10·69 ±4·36	0·11
Resistive index	0·66 ±0·07	0·71 ±0·14	0·87
Pulsatility index	1·45 ±0·33	1·81 ±1·12	0·84

t-Test for paired samples at 5% of significance level
*Significant for P<0·05

Using Power Doppler, the mean values of maximum velocity were 28·71 cm/s (confidence interval 95%: 25·51 and 31·91 cm/s) for malignant tumours and 19·91 cm/s (confidence interval 95%: 15·81 and 22·57 cm/s) for benign neoplasms (P=0·01). The values for sensitivity, specificity, PPV and NPV of maximum velocity were 65% (0·40 to 0·84), 100% (0·69 to 1·00), 100% (0·75 to 1·00) and 58% (0·32 to 0·81), respectively.

For minimum velocity, no difference was seen (P=0·11) between mean values of 10·69 cm/s for malignant and 7·17 cm/s for benign tumours. Furthermore, no differences were found for vascular resistance (P=0·87) or pulsatility (P=0·84) between the two groups, with averages of 0·71 (RI) and 1·81 (PI) for malignant and 0·66 (RI) and 1·45 (PI) for benign tumours (Table 2; Fig 2).

Immunohistochemistry (VEGF)

Regarding semi-quantitative values for VEGF on mammary neoplasms of female dogs, an average of 2·22 ±0·89 was found for malignant tumours and 1·66 ±0·91 for benign neoplasms (P=0·03; Fig 3).

A positive correlations were found between VEGF and maximum blood flow velocity, as well as between VEGF and presence of tumoural vascularisation (P=0·04 and r=0·3658; P=0·03 and r=0·3913, respectively).

DISCUSSION

A greater number of malignant neoplasms than benign tumours were found during the histological assessment (60 and 40%, respectively). This result differs from those of previous studies, where 50% of tumours were benign in female dogs (Queiroga and Lopes 2002, Carvalho 2006) but presumably simply reflect small differences in the sample population.

The data obtained in our study constitute important tools for epidemiological and clinical studies for each type of mammary neoplasm in female dogs. The classification used in the present study is in agreement with previous work (Misdrop and others 1999).

Ultrasound examination revealed no significant difference in margin regularity between the groups. This result differed from that of Bastan and others (2009), who found margin regularity in benign tumours and margin irregularity in malignant tumours. Other studies revealed that some macroscopic (presence of irregular margins) and microscopic (infiltrative and/or destructive tissue growth) characteristics may indicate malignancy of the tumour tissue (Misdrop and others 1999; Lana and others 2007). However, such findings were not observed in the current study.

The presence of heterogeneous echotexture was previously reported as indicative of malignancy in mammary tumours in dogs and other species (Nyman and others 2006b). These findings are in disagreement with those of the current study. Mammary ultrasound revealed that some benign tumours may show heterogeneous echotexture, especially those exhibiting hyperechogenic foci in the parenchyma, and malignant tumours may present homogeneous parenchyma echotexture.

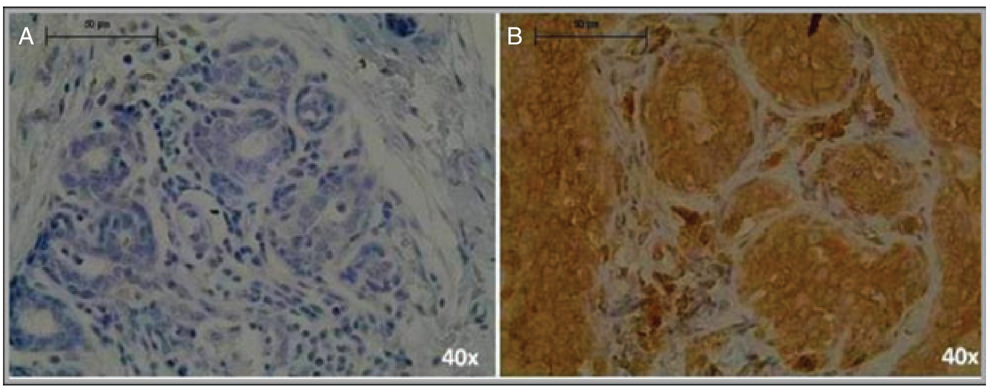

FIG 3. Pictures of semi-quantitative classification of VEGF of mammary tumours in female dogs. Note the absence of colour (score 1) in (A) (benign neoplasm) and the strong colour (score 4) in (B) (malignant neoplasm), at ×40 magnification

It was suggested that benign tumours with echogenic foci, characteristic of heterogeneity, are associated with specific types of tumours, particularly with those neoplasms containing bone, cartilage and/or fat tissue (Lana and others 2007). It is important to note that ultrasound with 12 MHz high resolution transducers was not used in other studies (Nyman and others 2006b; Bastan and others 2009). Detailed visualisation of smaller structures may be challenging without the use of high resolution ultrasound (Trasch and others 2007). In contrast, our study included the use of high resolution ultrasound, which enabled clear visualisation of neoplastic architecture.

Several factors are involved in the diagnosis of malignancy of neoplastic tissue (Misdrop and others 1999). Nevertheless, the absence of necrosis and/or tissue infiltration does not exclude malignancy. Necrotic areas usually appear as heterogeneous regions, but in the present study, ultrasound revealed that malignant tumours without such lesions exhibited homogeneous echotexture.

The visualisation of cystic areas in mammary neoplasms and other ultrasonographic findings, such as hyperechogenic foci, cannot be used to differentiate benign from malignant tumours. Such results support the conclusions of other studies (Nyman and others 2006b). Concerning the ultrasound findings in neoplastic tissue, the only alteration that could be used to differentiate malignant from benign tumours is the presence of acoustic shadowing. Further studies are required to evaluate the correlation of such ultrasound findings with diagnosis of mammary tumours in female dogs.

The use of colour-flow Doppler in the current study revealed that the presence of vascularisation in mammary tumours does not correlate with malignancy of neoplasms. This result contrasts with the findings of other studies (Nyman and others 2006b). A higher incidence of neovascularisation may be present in malignant tumours than in benign tumours. Visualisation of vessels depends on their size, which is a limitation for the imaging technique used previously (Nyman and others 2006b). In the current study, visualisation of smaller vessels was possible due to the high resolution ultrasound employed.

The peak systolic velocity (maximum velocity) found in mammary tumours can indicate tissue malignancy, as has been observed in humans (Dock and others 1991). Values obtained for malignant were significantly higher than for benign tumours, which is consistent with previous studies. There are no reports in the literature regarding minimum velocity, RI and PI that could aid in tumour diagnosis. Besides an increase in maximum velocity, the presence of turbulent flow is suggestive of malignancy (Lucena 2006). Nonetheless, such correlations were not found in the current study.

Despite the results obtained by Doppler mode assessment, it is important to note that in approximately 44% of malignant tumours and 54% of benign neoplasms, it was not possible to detect vascularisation using the Triplex Doppler, which limited the measurement of vascular index. The inability to detect vascularisation is an important limitation of this diagnostic technique in the differentiation of benign and malignant mammary tumours in bitches.

A previous study showed that alterations in tumoural blood flow detected by colour-flow Doppler can also be an indicator of tumour regression (Martins and others 2002). Therefore, Doppler examination can be important for the diagnosis of mammary neoplasms in female dogs and also for the assessment of drug treatments targeting the neoplastic blood supply.

The results obtained in the present study support those of Graham and Myers (1999) who proposed that VEGF is an indicator of tumoural diagnosis and prognosis of mammary neoplasms in dogs. In the present study, VEGF stimulated neovascularisation was observed in both benign and malignant tumours, as also stated by others (Restucci and others 2002, Lucena 2006).

An important result obtained was the correlation of maximum blood flow velocity with VEGF in tumoural tissue. The influence of VEGF on tumoural blood flow velocity has not been studied previously in veterinary medicine. The alterations caused by VEGF on the tumour endothelial wall (i.e. increased permeability, vessel dilation, bending to specific tyrosinase receptors of endothelial cells, stimulation of the growth of neoplastic cells and inhibition of apoptosis), as reported in other studies (Plate, 2001; Restucci and others 2002), may be responsible for the correlation found between VEGF and tumour blood flow velocity. Further studies are needed to clarify the correlation between VEGF and tumoural haemodynamics.

CONCLUSIONS

In conclusion, the use of Doppler ultrasound may be useful in differentiation of malignant from benign mammary neoplasms using vascular indices. However, the main limitation of this technique is the inability to detect blood flow in some mammary tumours. Conventional ultrasound in the bitches was not useful for the diagnosis of malignancy of mammary tumours, possibly because of the variety of tumour types found in dogs. There is a weak correlation between VEGF and tumoural tissue haemodynamics. Further studies are needed to verify the correlation between VEGF and malignant parameters such as mitotic figures, cytoplasmic ratio, growth rate and others.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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