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Original article

Diagnostic accuracy of spectral Doppler indices and shear wave elastography in dogs with mammary tumors and their relationship to tumor proliferation

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Abstract

The combination of several sonographic techniques can provide important information on pathological feature of mammary tumors. This study aims to evaluate the diagnostic accuracy of spectral Doppler indices and shear wave elastography (SWE) in dogs with mammary tumors and their relationship to tumor proliferation. The study consisted of malignant epithelial tumors (Group MET; n=49) and healthy mammary tissues (Group C; n=15). Ultrasonographic findings (echotexture, echogenicity, mass width, etc.), tissue Ki-67 levels, Doppler ultrasonography and sonoelastographic measurements were performed. Ultrasonography was performed by a single experienced veterinary sonographer prior to mastectomy using a Resona i9 ultrasonography device equipped with a linear transducer (L20-5s). Histopathological results were used as the gold standard. Tissue Ki-67 levels were measured using a canine Ki-67 Protein (Ki67P) ELISA kit. The distribution of echotexture and echogenicity in Group MET were mostly heterogeneous and hyperechoic. Also, the most frequent vascular pattern was a mixed pattern in Group MET, and the vascularization amount did not differ in terms of histological grades in canine mammary carcinomas. Vascular resistivity (RI) in Group MET was significantly higher than in Group C while pulsatility indexes (PI) was not significantly different between the groups . Peak systolic velocity (PSV) and end diastolic velocity (EDV) increased in malignant tumors and showed adequate sensitivity and specificity values to distinguish the malignant tumors than healthy mammary tissues. Shear wave speed and stiffness of malignant tumors were higher than healthy mammary tissue. The highest shear wave speed and tissue stiffness were observed in tumors larger than 5 cm. Elastography parameters in Grade 3 carcinomas were higher than in Grade 2 carcinomas. Tissue Ki-67 concentration was significantly associated with Doppler velocimetry parameters and SWE measurements. Also, Ki-67 was accurate in distingiuishing the healthy mammary tissue than canine mammary carcinomas with moderate sensitivity and high specificity. Tissue Ki-67, RI, PSV, shear wave speed, and stiffness amounts have 100% specificity to distinguish malignant mammary carcinomas compared to healthy mammary tissues in dogs. It was concluded that a combination of imaging techniques should be applied for a more accurate diagnosis of mammary tumors.

Keywords: canine mammary tumor, Doppler ultrasonography, Ki-67, shear wave elastography

Introduction

Mammary tumors are mostly malignant and are frequently seen tumor types in dogs. Canine mammary tumors are naturally occurring heterogenous group of cancers that have similar etiology, signaling pathway activation and histological classification features with human breast cancer (Sorenmo et al. 2009). An estimation of a tumor's proliferation potential is useful for determining tumor malignancy. A high proliferation rate is associated with rapid tumor growth and the ability to cause local and distant metastases. One of the widely used cell proliferation markers is antigen Ki-67 (Ki-67) (Kaszak et al. 2022). Rodrigues et al. (2016) indicate that the expression level of Ki-67 is positively correlated with adverse clinicopathological parameters and jointly signifies aggressive tumor behavior and poor prognosis in dogs with mammary tumors. However, Estaller et al. (2022) determined no significant difference in serum Ki-67 concentration between benign and malignant mammary tumours.

Non-invasive diagnostic ultrasound techniques such as B-mode ultrasound, Doppler technique and real time elastography are important requirements in both human and veterinary medicine (Kaszak et al. 2022). Even though the gold standard for the diagnosis of mammary lesions is the histopathological examination, some ultrasonographic features described, can predict the potential of a lesion being malignant (Feliciano et al. 2023). Esteves-Monteiro et al. (2025) reported that tumor size, shape, border irregularity, echotexture, internal echogenicity, and acoustic transmission parameters were useful for the evaluation of mammary gland tumors in dogs and cats. Doppler ultrasonography can be used for the estimation of blood flow into the mammary glands. Color flow imaging enables the visualization (present or absent) and localization (peripheral, central or diffuse) of tumoral vascularization present in tumors (Feliciano et al. 2023). The spectral Doppler measures peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI) and pulsatility index (PI) (Barbagianni and Gouletsou 2023). High vascularity and increases in spectral vascular indices are predictive of malignant masses (Uçar et al. 2021). Elastography is an ultrasonographic technique employed to measure the elasticity and stiffness of tissues, to increase diagnostic sensitivity. The elasticity of a tissue (even within the same organ) may vary according to the physiological state (e.g., lactating or dry mammary gland) or the pathological process (e.g., inflammation, neoplasia) in the tissue (Barbagianni and Gouletsou 2023). Sonoelastography methods were sufficiently able to distinquish malignant mammary tumors from benign ones (Feliciano et al. 2023, Şeran et al. 2024). It is based on the principle that malignant tissues are generally stiffer than benign ones (Esteves-Monteiro et al. 2025). The combination of several sonographic techniques can provide important information on pathological features of the mammary tumor (Feliciano et al. 2023).

Several studies have been carried out to evaluate the applicability of the ultrasonographic examination of mammary lesions in bitches. This study aims to evaluate the diagnostic accuracy of spectral Doppler indices and shear wave elastography in dogs with mammary tumors and their relationship to tumor proliferation.

Materials and Methods

Ethics statement

Permission for the study was approved by the Istanbul University-Cerrahpaşa Animal Experiments Local Ethics Committee (2023/10). The animal owners' permission was obtained in order to conduct the research.

Animal subjects and study design

The G-power test was applied to calculate the total sample size (G*Power version 3.1.9.7 software, Heinrich-Heine-Universität Düsseldorf, Germany). During this test, when the effect size was 0.80, α err prob: 0.05, and power (1-β err prob): 0.80, the minimum sample size for each group was set at n=14 to obtain statistical results in the two-group comparison, and the minimum total sample size for the study was set at n=28.

Thirty-three female dogs were presented to the Istanbul University-Cerrahpaşa Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology clinic between 2023 and 2025 with complaints of mammary masses. The dogs were medium sized breed, and their age range was 8-12 years. During the clinical examination, the two mammary chains and the lymph nodes were explored. Canine mammary neoplasms were commonly presented as circumscribed nodules with variable size, consistency, and mobility to the skin and muscle. The tumors associated with skin ulceration and local inflammatory reactions were not included in the study. Three-view thoracic radiography (DR, Ekovia, Korea) and total blood count (Catalyst One, IDEXX, USA) were performed to evaluate the preoperative anesthesia risks in all groups. Bilateral mastectomy was performed under general anesthesia for all dogs, as defined by Uçmak et al. (2021). General anesthesia was induced with 6 mg/kg of propofol (Pofol®, Dongkook, Korea) intravenously and followed by isoflurane (3%) (Foran liquid®, Abbott Laboratories, UK) in oxygen (2%).

All animals and a total of 81 masses were evaluated by ultrasonography (Resona i9, Mindray, China) and subsequently classified as benign, healthy or malignant by histopathology. As a result of histopathological examination, a total of 66 mammary tissue samples were included in the study. The study consisted of malignant epithelial tumors (Group MET; n=49) and healthy mammary tissues (Group C; n=15). The remaining 15 tissues had hyperplasia dysplasia (n=5), carcinosarcoma (n=6), and benign neoplasms (n=4) and were not included in the study due to low sample size. After mastectomy, tissue samples were collected (0.5cm per two Eppendorf tubes) and stored at -86°C until ELISA analysis was performed.

Ultrasonography examination

Ultrasonography (US) was performed by a single experienced veterinary sonographer prior to mastectomy using a Resona i9 US device equipped with a linear transducer (L20-5s) (Mindray, China). Each mammary mass was evaluated using the different ultrasonographic methods (B-mode, Doppler, shear wave elastography (SWE)) in the order described below.

B-mode ultrasonography

The mammary masses were evaluated by conventional ultrasonography according to: echotexture (homogenous or heterogeneous), echogenicity (hypoechoic, hyperechoic, anechoic or mixed with solid or liquid components), contours/ margins (defined or undefined) and invasiveness (present or absent). Masses with cystic structure were excluded from the study. Additionally, width (cm) of the mammary tissue in transverse sections was obtained. Mass sizes were classified according to the TMN staging system (T1<3 cm, T2: 3-5 cm, T3> 5 cm) (Owen 1980).

Doppler ultrasonography

Power Doppler imaging enabled the visualization (present or absent) and localization (peripheral, central or diffuse) of tumoral vascularization. The amount of vascularization (color pixel percentage; CPP%) was automatically calculated by the software of the US device. In tumoral vascularization analysis by spectral Doppler, the angle between the Doppler beam and the vessel's long axis did not exceed 60°. A 2-4 mm gate (depending on the diameter of the vessel) with apertures was positioned at the center of the vessel to measure the flow's spectral trace, spectral curve and vascular indices, which were obtained automatically following software identification of the ultrasonic scanner for each waveform. A minimum of three subsequent waves was used in the evaluation. The parameters studied were peak systolic velocity (PSV; cm/s), end diastolic velocity (EDV; cm/s), resistive index (RI) and pulsatility index (PI).

Shear wave elastography

For each mass that had completed B-mode and Doppler ultrasonography examinations, the US device's 2D shear wave elastography module was activated. During SWE, after the motion stability index (MSI) reached ≥4 stars, a shear wave was delivered to the tissue. At this stage, images with a reliability index of >85% were recorded, and quantitative measurements were made from these images. A qualitative map is created on the screen based on the stiffness levels of the tissues in the shear wave area; red represents the stiffest tissue, and blue represents the softest. For quantitative measurements, at least two regions of interest (ROIs) were determined from each recorded image, and each ROI was set to 2 cm in diameter. Quantitative measurements were performed automatically by the device. The tissue stiffness is presented as minimum, maximum, average and standard error values based on the measurement of each ROI. Two measurements were taken from each mammary lobe, and the average of these measurements was calculated to determine tissue stiffness. As a result of elastography measurements, the stiffness of the tissue was obtained in kilopascals (kPa) and the speed of the shear wave passing through the mammary tissue was obtained in m/s.

Histopathological classification

After tissue section samples were taken, the remaining total mastectomy material was sent to the pathology department for histopathological analysis in 10% formaldehyde solution. Mammary tumor tissues were fixed with 10% buffered formalin solution for 48 hours. Samples were then subjected to routine tissue processing procedures, paraffin blocks were prepared and 5 µm thick sections were cut and stained with Hematoxylin & Eosin (H&E). Sections were examined under a light microscope (Nikon Eclipse Ci-L, Japan) and each case was labelled according to the canine mammary tumor classification of Goldschmidt et al. (2011) and graded according to the grading system of Pena et al. (2013). Histopathological results were used as the gold standard. The histopathological appearance of solid carcinoma, mixed type carcinoma, complex type carcinoma, and intraductal papillary carcinoma are presented at Fig. 1.

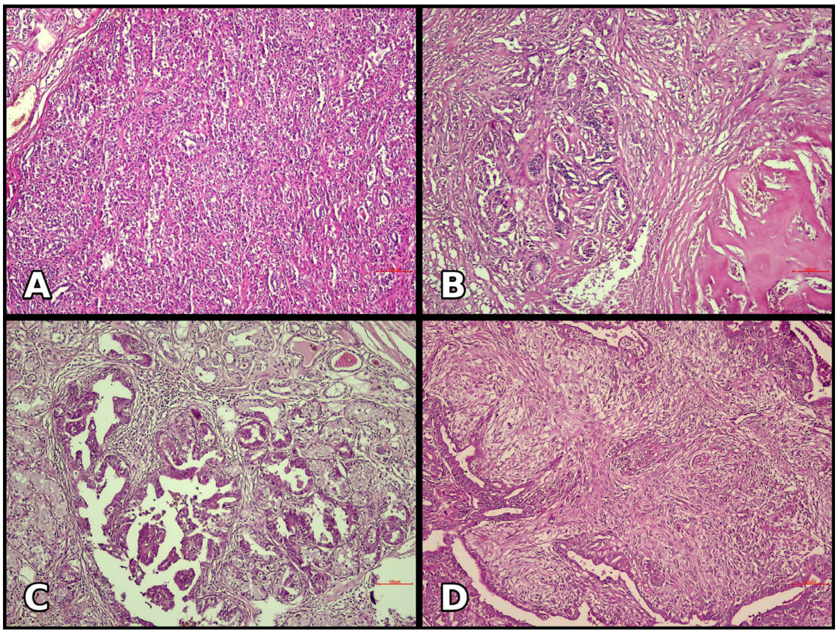

Fig. 1. Histopathological appearance of carcinoma subtypes in dogs with mammary tumors. **A.** Solid carcinoma. Neoplastic epithelial cells arranged in densely packed solid sheets exhibiting moderate anisokaryosis and a high nuclear density (H&E, scale bar = 100 µm). **B.** Carcinoma, mixed type. Neoplastic epithelial cells forming irregular tubulopapillary structures within a prominent fibromyxoid stroma containing areas of chondroid matrix production (H&E, scale bar = 100 µm). **C.** Intraductal papillary carcinoma. Neoplastic epithelial cells form multilayered papillary projections supported by fibrovascular cores within dilated ducts (H&E, scale bar = 100 µm). **D.** Carcinoma, complex type. Malignant epithelial cells admixed with proliferative spindle-shaped myoepithelial cells (H&E, scale bar = 100 µm).

ELISA analysis

Tissue samples were diluted to ensure homogeneity. For this purpose, the tissues were weighed and cut into small pieces using a sterile scalpel. Approximately 100 mg of tissue was diluted with 1 ml (1000 µl) of Phosphate Buffered Saline (PBS). The tissues were homogenized in a cooling container using a homogenization blender (Miccra-D1, Miccra, Germany). The homogenate was centrifuged at 3000 rpm for 20 minutes (Sigma 3K30, Sigma Laborzentrifugen GmbH, Germany). The supernatant was collected and transferred to a separate Eppendorf tube. Tissue homogenates were used for ELISA analysis (Bio-Tek, USA). Tissue Ki-67 levels were measured using a canine Ki-67 Protein (Ki67P) ELISA kit according to the manufacturers' instructions (Canine Ki-67 Protein ELISA Kit, Cat.No: MBS089640, Mybiosource, USA). The detection range of Ki-67 was 0.625 ng/ml-20 ng/ml. The intra and inter assay coefficients of variation were 4.82% (n=5) and 8.34% (n=5), respectively.

Statistical analysis

Statistical analysis was performed by using SPSS 31.0 (IBM, USA). Normal distribution of data and homogeneity of variances were evaluated using the Shaphiro Wilk test and Levene's test, respectively. The comparison of groups in terms of Ki-67, CPP%, Doppler and elastography measurements was performed using Mann-Whitney U test. However, changes in RI values between the groups were evaluated using Student's t-test. In Group MET, comparision of histological grades and mass sizes in terms of evaluated parameters were performed using OneWay Anova. The relationships among the parameters were determined with the Pearson correlation test. The receiver-operating characteristics (ROC) curve analysis was performed to determine the sensitivity and specificity of Ki-67, CPP%, Doppler and elastography parameters. Areas under the ROC curves (AUC) were assessed to determine the discrimination ability in dogs with mammary carcinomas or healthy ones. Values were given as mean ± standard error of the mean (SEM). The significance level was accepted as $p<0.05$.

Results

The mean age of the 33 dogs was 10.41±0.38 years. On clinical examination, sizes of the mammary neoplasms ranged from 1 to 7 cm in the widest cross-sectional diameter. Multiple tumors were frequently observed in one mammary chain. The existence of malignant mammary tumors was more frequently at the caudal abdominal and inguinal mammary glands than at the thoracic glands. According to histopathology, malignant epithelial tumor was diagnosed in 49 of 66 mam-

Table 1. B mod US, Doppler US and sonoelastographic measurements, tissue Ki-67 levels and their comparison between the dog groups.

			Group MET (n=49)	Group C (n=15)	Significance
B mod US Findings	Echotexture	Homogenous	27	12	
		Heterogeneous	22	3	
	Echogenicity	Hyperechoic	27	3	
		Hypoechoic	22	12	
	Structure	Solid	24	-	
		Multilobulated	12	-	
		Diffuse	13	-	
Doppler US Findings	Vascularization	Peripheric	19	9	
		Central	3	3	
		Mixed	27	3	
	CPP %		7.36±0.86[a]	3.43±0.63[b]	=0.01
	PI		1.97±0.12	2.58±0.33	>0.05
	RI		0.78±0.01[a]	0.69±0.02[b]	<0.05
	EDV (cm/s)		3.12±0.43[a]	1.33±0.39[b]	<0.05
	PSV (cm/s)		13.12±0.43[a]	7.12±0.61[b]	<0.001
SWE Findings	EkPa (mean)		57.94±7.07[a]	28.61±2.38[b]	<0.01
	Cs (m/s, mean)		4.05±0.20[a]	3.03±0.15[b]	<0.01
Tissue Ki-67			6.85±0.20[a]	5.68±0.12[b]	<0.001

[a,b] Different superscript letters in the same row indicate the significance. CPP – Color pixel percentage, PI – Pulsatility index, RI – Resistive index, PSV – peak systolic velocity (cm/s), EDV – end diastolic velocity (cm/s), EkPa – Stiffness, Cs – Shear wave speed

mary tissues. Ultrasonographic findings (echotexture, echogenicity, mass width, etc.), tissue Ki-67 levels, Doppler US and sonoelastographic measurements and their comparison between the groups are presented in Table 1. Mammary lobes with anechoic and cystic structure were not included in this study. Mammary tumors in Group MET mostly had homogenous echotexture, hyperechoic echogenicity and solid structure. Mixed vascularization mostly existed in Group MET, while peripheric vascularization was in Group C. Doppler US measurements revealed increased CPP, RI, EDV, and PSV values in Group MET. Also, stiffness of the tumors in Group MET were higher compared to healthy mammary tissues. Group MET was also classified in terms of mass size and histological gradings: thirty mammary tumors were T1 size, nine tumors were T2 size and ten tumors were T3 size; according to histopathological examination, 18 tumors were Grade 1, 19 tumors were Grade 2 and 12 tumors were Grade 3. The histopathological distribution of mammary tumors in Group MET was simple carcinoma (n=14), solid carcinoma (n=6), complex carcinoma (n=7), mixed carcinoma (n=9), intraductal carcinoma (n=6) and carcinoma malignant myoepithelioma (n=7). In solid carcinoma cases, neoplastic epithelial cells were arranged in densely packed solid sheets exhibiting moderate anisokaryosis and a high nuclear density (Fig. 1A). In mixed type carcinoma cases, neoplastic epithelial cells formed irregular tubulopapillary structures within a prominent fibromyxoid stroma containing areas of chondroid matrix production (Fig. 1B). Intraductal papillary carcinoma cases exhibited neoplastic epithelial cells in multilayered papillary projections supported by fibrovascular cores within dilated ducts (Fig. 1C). In complex type carcinoma cases, malignant epithelial cells admixed with proliferative spindle-shaped myoepithelial cells (Fig. 1D). Changes of evaluated parameters among the histological gradings and mass size in Group MET are shown in Table 2. CPP in T2 sized tumors were higher than in T1 sized tumors ($p<0.05$). Also, the highest elasticity measurements and Ki-67 values were recorded in T3 sized tumors. Doppler indices (PI and RI) in grade 3 tumors were higher compared to grade 2 tumors ($p<0.05$). However, the highest EDV and PSV values were obtained in grade 2 tumors. Also, elasticity parameters in grade 3 tumors were higher than in Grade 2 tumors while Ki-67 levels in Grade 2 tumors were higher than in grade 1 tumors. The relationships among the tissue Ki-67 levels, Doppler and elastography parameters are given in Table 3. Tissue Ki-67 levels were positively correlated with CPP ($p<0.05$), Doppler velocities (PSV and EDV; $p<0.01$) and elastography parameters (EkPa, Cs) ($p<0.05$). However, significant associations, particularly those between Ki-67 and CPP%, elastography parameters were weak to moderate in strength ($r \approx 0.25$-0.27). Also, PI value was inversely correlated with CPP ($p<0.01$), PSV ($p<0.05$), and EDV ($p<0.001$), while PI was significantly associated with

Table 2. Changes of evaluated parameters among the histological gradings and mass size in Group MET.

Parameters	T1 (<3 cm)	T2 (3-5 cm)	T3 (>5 cm)	Significance	
CPP %	5.03±0.46[a]	14.21±3.18[b]	8.22±1.59[ab]	<0.05	
PI	1.94±0.16	1.59±0.21	2.12±0.24	>0.05	
RI	0.77±0.02[ab]	0.73±0.05[a]	0.87±0.03[b]	<0.05	
EDV (cm/s)	2.25±0.31	4.42±0.82	4.43±1.58	>0.05	
PSV (cm/s)	10.96±0.86	15.05±2.27	16.90±4.44	>0.05	
EkPa (mean)	39.19±3.02[a]	44.94±5.24[a]	126.30±23.34[b]	T1 vs T3 <0.001	T2 vs T3 <0.01
Cs (m/s; mean)	3.52±0.14[a]	3.69±0.23[a]	5.99±0.58[b]	T1 vs T3 <0.01	T2 vs T3 <0.01
Ki-67	6.49±0.12[a]	6.36±0.25[a]	8.34±0.74[b]	T3 vs T1 <0.01	T3 vs T2 <0.05
Parameters	Grade 1	Grade 2	Grade 3	Significance	
CPP %	6.37±1.65	8.63±1.23	6.85±1.58	>0.05	
PI	1.79±0.13[ab]	1.55±0.13[a]	2.58±0.33[b]	<0.05	
RI	0.71±0.02[ab]	0.73±0.03[a]	0.86±0.03[b]	<0.05	
EDV (cm/s)	2.28±0.36[a]	4.89±0.86[b]	1.57±.39[a]	<0.05	
PSV (cm/s)	10.64±1.01[a]	17.17±2.48[b]	9.77±0.93[a]	<0.05	
EkPa (mean)	49.12±4.17[a]	44.53±10.74[b]	92.40±20.22[a]	G1 vs G2 <0.05	G3 vs G2 <0.01
Cs (m/s; mean)	4.00±0.19[a]	3.49±0.31[b]	5.02±0.53[a]	G1 vs G2 <0.05	G3 vs G2 <0.01
Ki-67	6.24±0.12[a]	7.17±0.36[ab]	7.23±0.52[b]	<0.05	

[a,b] Different superscript letters in the same row indicate the significance. CPP – Color pixel percentage, PI – Pulsatility index, RI – Resistive index, PSV – peak systolic velocity (cm/s), EDV – end diastolic velocity (cm/s), EkPa – Stiffness, Cs – Shear wave speed

Table 3. Relationships among the evaluated parameters (Pearson correlation coefficients and their significance).

Parameters	Ki-67	CPP %	PI	RI	PSV	EDV	Ekpa	Cs (m/s)
Ki-67	–	0.268*	-0.095	0.149	0.451***	0.400**	0.254*	0.270*
CPP %		–	-0.350**	-0.201	0.353**	0.398**	-0.059	-0.043
PI			–	0.561***	-0.248*	-0.528***	-0.016	0.058
RI				–	0.013	-0.338**	0.347**	0.394**
PSV (cm/s)					–	0.832***	0.018	0.014
EDV (cm/s)						–	0.057	0.018
EkPa							–	0.953***
Cs (m/s)								–

CPP – Color pixel percentage, PI – Pulsatility index, RI – Resistive index, PSV – peak systolic velocity (cm/s), EDV – end diastolic velocity, EkPa – Stiffness, Cs – Shear wave speed, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

RI ($p<0.05$). However, weak association (r=0.248) was detected between PI and PSV values. A graphical view of the ROC curve is presented in Fig. 2. The sensitivity and specificity of Ki-67, Doppler and elastography parameters were determined and are presented in Table 4.

Discussion

Ultrasonography could be used as a noninvasive method in the examination of the canine mammary gland, as it is in humans (Baştan et al. 2009). Ultrasound examination has highlighted the existence of a characteristic architecture of the layers of healthy mammary glands in female dogs. These aspects could easily be seen both using a microconvex transducer and a linear transducer (Balaci et al. 2015). Similarly,

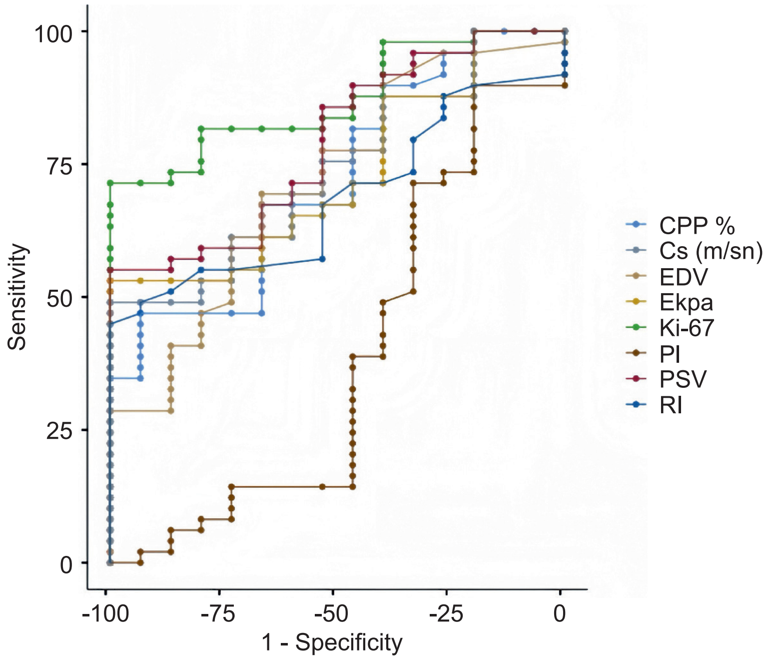

Fig 2. Graphical view of Receiver Operating Characteristic (ROC) curves for all parameters. CPP %: Color pixel percentage, PI: Pulsatility index, RI: Resistive index, PSV: peak systolic velocity (cm/s), EDV: end diastolic velocity (cm/s), EkPa: Stiffness, Cs: Shear wave speed

Table 4. Receiver-operating characteristics (ROC) curve analysis results.

Parameters	Sensitivity %	Specificity %	AUC	Cut Point	Youden's index
Ki-67	71.43	100	0.872	6.228	0.714
CPP %	46.94	93.33	0.722	6.42	0.403
PI	89.8	20	0.384	1.03	0.098
RI	44.9	100	0.686	0.82	0.449
PSV (cm/s)	55.1	100	0.789	10.51	0.551
EDV (cm/s)	69.39	66.67	0.716	1.56	0.716
EkPa	53.06	100	0.731	38.32	0.731
Cs (m/s)	48.98	100	0.739	3.865	0.739

CPP – Color pixel percentage, PI – Pulsatility index, RI – Resistive index, PSV – peak systolic velocity (cm/s), EDV – end diastolic velocity (cm/s), EkPa – Stiffness, Cs – Shear wave speed

a linear transducer was used to evalute the mammary lobes in this study. Healthy mammary tissue is visible on the ultrasound image as a middle layer of heterogenous echotexture with intermittent hyper-echoic and hypo-echoic areas. The muscular layer is the most profound layer of medium echogenicity which is separated by a thin hyper-echoic layer of abdominal fascia (Thomas et al. 2022). Balaciet al. (2015) reported that mammary parenchyma had coarse-grained structure with medium echogenicity. In this study, mammary parenchyma in healthy mammary tissues mostly had homogenous echotexture and hypoechoic echogenicity. Canine mammary tumors with a varied echogenicity (heterogenous echotexture) are likely to be malignant (Baştan et al. 2009). Benign tumours had mostly homogeneous echotexture and malignant tumours showed a higher frequency of heterogeneity (Soler et al. 2016). Also, malignant mixed tumors had higher homogeneity and contrast compared to benign mixed tumors (Mülazımoğlu et al. 2016). The distribution of echotexture and echogenicity in Group MET were similar and a mostly heterogeneous and hyperechoic view existed in malignant mammary tissues. Using B-mode ultrasonography, tumor shape can be used to identify masses in the mammary gland, and tumors can be classified as round, oval, lobed or irregular. Studies have shown that benign human breast tumors are generally round or oval with smooth borders, while malignant mammary tumors are often irregular or multilobed (Uçar et al. 2021). An irregular tumor border indicates an increased probability that the tumor is malignant (Baştan et al. 2009). Thomas et al. (2022) indicated that malignant mammary neoplasms have an undefined margin on B-mode ultrasonography. However, regular and multilobed mammary tumors were dense in Group MET and undefined, diffuse malignant mammary tumors were also found in this study.

Doppler US, used to examine mammary masses, provides more sensitive results than B-mode imaging (Feliciano et al. 2018). One of the features evaluated in studies conducted with Doppler ultrasonography on mammary tumors in dogs is the presence of vascularization and the localization of vascularization (central, peripheral, mixed) (Thomas et al. 2022). Feliciano et al. (2017) found that 67% of malignant mammary tumors had vascularization, and the location of the vascularization was peripheral in 75% of cases. Soler et al. (2016) reported that the most common vascular pattern in malignant tumors was the mixed pattern, while it was the peripheral pattern in benign tumors. Similarly to Soler et al. (2016), the most frequent vascular pattern was observed in Group MET and was the mixed pattern. Other research (Thomas et al. 2022) indicates that malignant tumors induce an abnormal vascular supply and have greater small vessels throughout the tumor due to strong angiogenesis. As previously reported, the increased CPP% in Group MET is due to strong angiogenesis in malignant tumors. Similarly to Feliciano et al. (2018), the vascularization amount did not differ in terms of histological grades in canine mammary carcinomas.

Azhar et al. (2023) reported that an increase in the value of the RI indicates an increase in the risk of malignancy in human breast cancer. Youssefzadeh et al. (1996) noted higher resistive indices in carcinomas than in normal tissue, since their continuous growth leads to an increase in tissue pressure with eventual reduction of distal flow. In contrast, vascular resistivity and pulsatility indices were not significantly different in benign and malignant canine mammary gland tumors (Soler et al. 2016, Feliciano et al. 2017). Similarly to the studies on human breast cancer but in contrast to studies on canine mammary tumors, the highest RI was observed in Group MET. However, PI values did not differ between the groups in this study regarding malignancy, as previously reported (Soler et al. 2016, Feliciano et al. 2017, Thomas et al. 2022). Feliciano et al. (2017) reported that color and spectral Doppler variables did not provide any data that could be used in the identification of high-grade mammary carcinomas in dogs. In our study, the extent of vascularization did not differ according to histologic grade, as malignant epithelial tumors showed signs of neovascularization across all grades of malignancy. However, contrary to the previous report (Soler et al. 2016, Feliciano et al. 2017, Thomas et al. 2022), Doppler indices (PI and RI) in Grade 3 tumors were higher than in Grade 2 tumors, which are reported as indicators of malignancy. In addition, the increase in RI values in tumors larger than 5 cm in our study is supported by the report of Sorenmo et al. (2009), which states that there is a strong relationship between tumor size and malignancy.

High vascularity on color Doppler imaging and increases in spectral vascular indices such as systolic velocity (SV) and diastolic velocity (DV) are predictive of malignant masses (Feliciano et al. 2017). The peak systolic velocity (maximum velocity) found in mammary tumors can indicate tissue malignancy in humans (Dock et al. 1991) and dogs (Feliciano et al. 2012). Vascular index values are higher in malignant mammary tumors compared to benign mammary tumors (Feliciano et al. 2017). Also, Thomas et al. (2022) reported that elevated peak systolic velocity of blood flow within the tumor vessels in malignant mammary neoplasms was the only feature assessed with Doppler mode to have a significant difference. Similarly to previous reports, Doppler spectral vascular indices PSV and EDV were greater in malignant tumors and showed adequate sensitivity and specificity values as being malignancy predictors on canine mammary masses in this study. The findings could be explained by Dock et al. (1991) who state that the absence of non-striated muscle structure seen in normal vessels leads to an abnormal course and distribution of tumor vessels, which alters the blood flow velocity and appears as accelerated flow on Doppler ultrasonography.

Feliciano et al. (2018) reported that Doppler velocimetry values did not differ according to mammary carcinoma types and their grades in dogs. In contrast with this previous report, the highest EDV and PSV values were observed in Grade 2 tumors, while velocimetry parameters were not influenced by the difference in tumor sizes, in our study. Based on the hypothesis of Folkman et al. (1989) on neovascularization in tumors with great growth potential, newly formed vessels associated with carcinoma are responsible for the features of Doppler waveform signals. Doppler velocities (PSV and EDV) were greater in malignant tumors, showing average sensitivity 79.2%, specificity 70.8%, accuracy 71.6% and AUC 73.0% in canine mammary tumors (Feliciano et al. 2017). In our study, the very strong correlations observed between PSV and EDV ($r=0.832$, $p<0.001$) demonstrated the synegystic effect of these parameters as malignancy predictors for canine mammary masses.

Elastography is an ultrasonographic technique used to measure stiffness of tissues, in order to increase diagnostic sensitivity (Ophir et al. 1991). SWE, can provide quantitative elastic information in both kPa and m/s. To convert units from Young's modulus elasticity (E) in kilopascal and shear wave speed (Cs) in m/s are directly linked through the simple formula: E=3G= 3 p Cs^2 (Sigrist et al. 2017). Consistent with the definition in the mathematical formula, a very strong correlation was observed between EkPa and Cs ($r=0.953$, $p<0.001$) in this study. The elastography of proliferative lesions of the mammary gland is a useful diagnostic method for distinguishing benign neoplastic lesions from malignant neoplasms (Glińska-Suchocka et al. 2013, Feliciano et al. 2017). Glińska-Suchocka et al. (2013) observed that benign neoplasms of the mammary gland showed low stiffness, whereas malignant neoplasms were characterized by high stiffness. However, Massimini et al. (2022) determined similar stiffness with malignant mammary nodular lesions and hyperplastic/benign neoplastic lesions in dogs. In our study, Shear wave speed and stiffness in malignant tumors were higher than in healthy mammary tissue. The increased stiffness of malignant lesions may be explained by the presence of a stromal reaction induced by mammary carcinoma associated with increased amounts of collagen components in tumor tissues (Feliciano et al. 2015). These results suggest that SWE evaluations may be helpful in predicting malignancy in canine mammary masses with moderate sensitivity and high specificity.

Massimini et al. (2022) reported that the accuracy of elastography appears to be limited in differentiating between benign and malignant canine mammary lesions, as well as discriminating between the different histological subtypes or grades of malignancy. Also, Şeran et al. (2024) reported that elasticity score and strain ratio of the malignant epithelial tumors in different grade groups were not statistically different. However, Feliciano et al. (2018) stated that acoustic radiation force impulse–elastography enabled the detection of grade II and III carcinomas with moderate accuracy. In contrast with previous reports, Shear wave speeds and tissue elasticity in Grade 3 carcinomas were higher than in Grade 2 carcinomas in our study. Pieczewska et al. (2021) evaluated the density of the canine mammary tumor by SWE before and after the treatment with aglepristone and found no influence on the density of the tumor tissue, although significant reduction in the mammary tumor's size was observed following treatment. However, Youk et al. (2013) indicated that the mean elasticity value was correlated with larger size and higher histological grade in invasive breast cancer patients. Similarly, in our study, the highest shear wave speed and elasticity were determined in tumors larger than 5 cm. Similar results obtained in human breast cancer cases confirm the clinicopathological similarity of canine mammary tumors to human breast cancers (Sorenmo et al. 2009).

Ki-67 is a nuclear antigen found in cells in the proliferative phase of the cell cycle, by which tumor proliferation is measured. Estaller et al. (2022) reported that serum Ki-67 concentration in dogs with malignant tumors was higher than in healthy dogs. Although tissue Ki-67 concentration was evaluated in this study, similar results were obtained as those by Estaller et al. (2022). Tissue Ki-67 concentration was significantly associated with Doppler velocimetry parameters and SWE measurements in our study. Also, Ki-67 was accurate in predicting malignancy in canine mammary masses with moderate sensitivity and high specificity. This suggests that the relationship between the mean elasticity value and Ki-67 is not independent, and the relationship between Ki-67 and the mean elasticity value may be due to the relationship between the mean elasticity and histological grade or vascular invasion (Youk et al. 2013). In our study, tissue Ki-67, RI, PSV, Shear wave speed, and stiffness amounts have 100% specificity in distinguishing the malignant mammary tumors compared to healthy mammary tissues in dogs.

In our study, in addition to pairwise comparison tests and ROC analysis, we also performed correlation analysis to determine the relationship between the parameters. However, correlation does not imply causality. This can be considered a limitation of the study. The correlation analysis is particularly relevant for associations between Ki-67, vascularization parameters and tissue stiffness, which may be influenced by confounding factors such as tumor size, histological subtype, or grade.

Conclusion

We concluded that a combination of imaging techniques can be applied for a more accurate diagnosis of mammary tumors. In future studies, the effectiveness of drug treatments in canine mammary tumors can be evaluated with the support of a combination of modern ultrasound techniques.

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Author Declarations

Ethics approval

Permission for the study was approved by the Istanbul University-Cerrahpaşa Animal Experiments Local Ethics Committee (2023/10). The animal owners' permission was obtained in order to conduct the research.

Use of generative artificial intelligence

Generative artificial intelligence tools were not used in the preparation of the manuscript.

Conflict of interest

The authors declare no competing interests.

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