

Accuracy of four ultrasonography techniques in predicting histopathological classification of canine mammary carcinomas

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Abstract

Due to the importance of presurgical, noninvasive, and accurate diagnostic tools in mammary carcinoma characterization, this prospective secondary observational cohort study was designed to evaluate and compare the diagnostic accuracy of B-mode, Doppler, contrast enhancement ultrasonography, or acoustic radiation force impulse–elastography in identifying mammary carcinomas types with high degree of malignancy. A total of 246 mammary carcinomas from 141 female dogs were analyzed using B-mode, Doppler, contrast enhancement ultrasonography, and acoustic radiation force impulse ultrasonography prior to their histopathological classification according to types (simple, complex, or special) and grade (I, II, or III). Qualitative and quantitative variables were compared between carcinoma types and grades by Fisher's or analysis of variance. Diagnostic performance was estimated by receiver-operating characteristic analysis, using histopathological classification as a reference. Deformability (acoustic radiation force impulse) had a diagnostic specificity of 100% and sensitivity of 12% in identifying special carcinomas. A width:length ratio greater than 0.53 can be suggestive of special carcinoma, with 80% sensitivity and 76% specificity. Contrast wash-in and peak enhancement times lower than 7.5 and 13.5 s, respectively, were indicative of complex carcinoma at 62% sensitivity and 60% specificity. Contrast wash-in, peak enhancement, and wash-out times greater than 6.5, 12.5, and 64.5 s, respectively; were indicative of grade II and III carcinoma at 68% sensitivity and 62% specificity. In conclusion, B-mode ultrasonography, contrast enhancement ultrasonography, and acoustic radiation force impulse–elastography enabled the identification of some of the characteristics of high-grade mammary carcinoma types and grades in female dogs with limited accuracy. The findings from this study may contribute to oncology research and clinical management canine patients.

KEYWORDS

canine, contrast-enhanced ultrasound, Doppler, elastography, tumors

1 | INTRODUCTION

Mammary tumors in female dogs are of great clinical relevance due to their high prevalence and mortality rate, which varies with the histopathological classification and graduation of the tumor.^{1,2} The clinical–surgical approach and prognosis may vary with tumor types and grade; therefore, the use of noninvasive presurgical accurate diagnostic techniques that enable the differentiation of these tumors is a clinical strategy with great diagnostic,

prognostic, and therapeutic value in patients with malignant mammary neoplasms.³

Ultrasound examination of mammary masses has been recently considered an important clinical tool in the identification of malignant neoplasms in female dogs. It has been suggested that B-mode ultrasonography could predict tumoral malignancy through heterogeneous echotexture; however, the accuracy of the reports was unclear. Mammary mass depth : width ratio obtained by B-mode ultrasonography resulted in 56.3% sensitivity and 92.9% specificity

in predicting malignancy. Similarly, Doppler mode ultrasonography reported 65% sensitivity and 100% specificity in predicting malignancy. Acoustic radiation force impulse elastography was considered a promising technique in determining tumor malignancy. On the other hand, contrast-enhanced ultrasonography enables the evaluation of capillarization evaluation but not malignancy differentiation.^{2,4-6}

A recent study involving 153 female dogs and 300 mammary masses evaluated and compared the efficacy of the ultrasound methods currently available, B-mode, Doppler, contrast enhanced ultrasonography, and elastography-acoustic radiation force impulse in predicting tumor malignancy. B-mode measurements showed 67.9% sensitivity and 67.6% specificity; Doppler, 79.2% sensitivity and 70.8% specificity;

contrast enhancement ultrasonography, 80.2% sensitivity and 16.7% specificity; and acoustic radiation force impulse shear wave velocities, 94.7% sensitivity and 97.2% specificity.⁷

To date, no study has attempted to correlate ultrasound examination and the histopathological type and/or grade of canine mammary carcinoma. Due to the importance of these diagnostic approaches in improving the prognosis and treatment of female dogs affected by mammary tumors, it was hypothesized that B-mode, Doppler, contrast enhancement ultrasonography, and elastography-acoustic radiation force impulse ultrasound evaluation can assist in noninvasive presurgical and identification of high-grade canine mammary carcinomas and, thus, provide immediate results for a more specific



FIGURE 1 B-mode ultrasonography image of malignant adenomyoepithelioma (special mammary carcinoma) in a female dog (arrows). Longitudinal section of the mammary lesions for length and width measurements [Color figure can be viewed at wileyonlinelibrary.com]

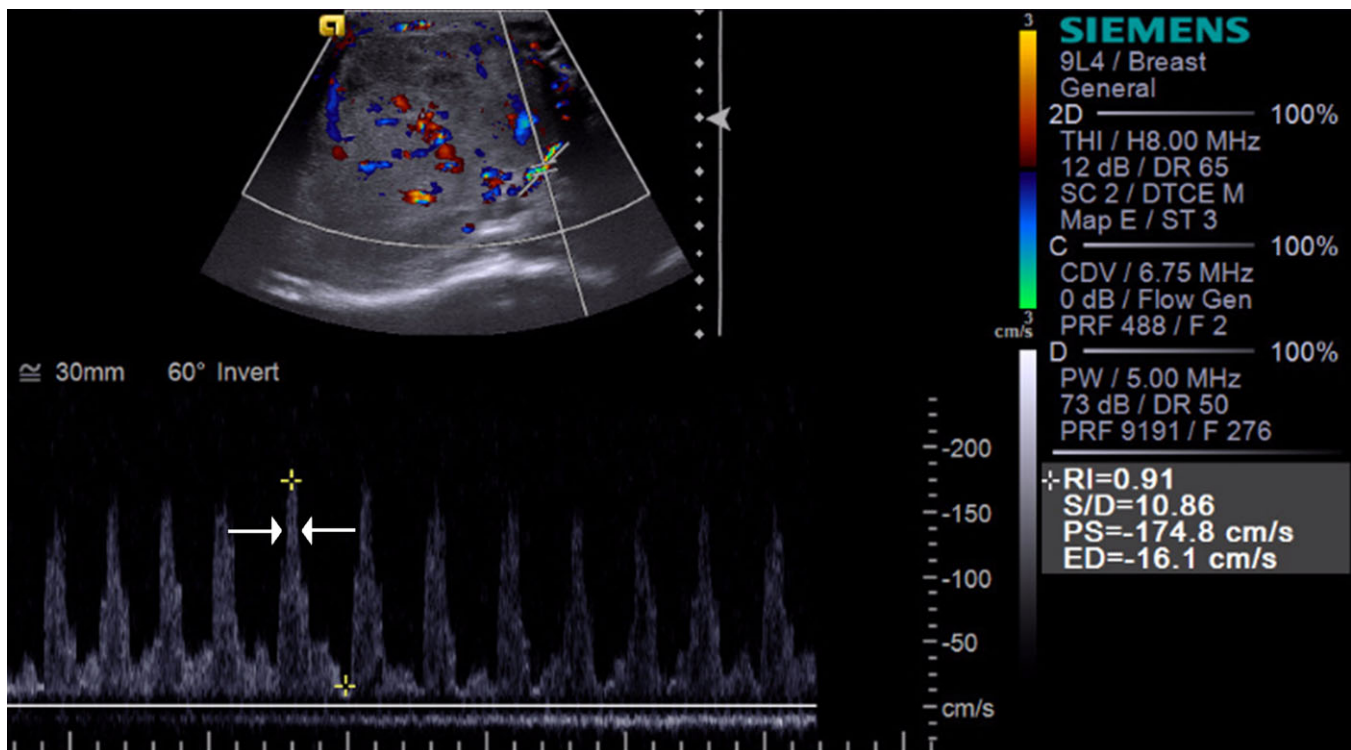


FIGURE 2 Doppler ultrasonography image of mammary carcinoma (grade I) in a mixed tumor in a female dog. Spectral Doppler ultrasonography image of mass with tumoral neovascularization and waveforms (broken arrow) blood flow of malignant lesion [Color figure can be viewed at wileyonlinelibrary.com]

clinical–surgical intervention. Thus, the aim of this study was to evaluate and compare the diagnostic accuracy of these ultrasound methods in the identification of mammary carcinoma types with a high degree of malignancy in female dogs with naturally occurring mammary neoplasms.

2 | METHODS

This study was a prospective secondary observational cohort design and was in keeping with the guidelines set out by the Control of Animal Experimentation. The primary study was conducted during the period of 2014–2016 and approved by the Ethics Committee in the Use of Animals of the Universidade Estadual Paulista, Jaboticabal–São Paulo, Brazil (protocol 023705/12). Free and informed consent was obtained from the owners prior to the animals being included in the primary study. For this secondary study, the data from physical and ultrasound examinations prior to mastectomy and histopathological classification of the canine mammary masses diagnosed as carcinomas in the primary study were retrieved and reanalyzed.⁷ A total of 246 mammary carcinomas were analyzed. The tumor clinical

characteristics evaluated were as follows: location, growth time, palpation consistency, and surface area.

As part of the inclusion criteria for this secondary study, ultrasonography for each included dog had been carried out by a single experienced sonographer (15 years), using virtual touch system, contrast agent technology, and a linear array 4–18 MHz transducer (Acuson S2000TM, eSie TouchTM, and CadenceTM; Siemens, Munich, Germany) according to the methodology previously described.⁷ The variables analyzed by B-mode ultrasonography were echotexture (homogenous or heterogeneous); contours/margins (defined or undefined); invasiveness (present or absent); length (cm), width (cm), and width:length ratio in longitudinal sections; and height (cm), width (cm), and width:height ratio in transverse sections (Figure 1). The variables analyzed by Doppler mode ultrasonography were vascular visualization by color flow (present or absent), systolic velocity (cm/s), diastolic velocity (cm/s), and resistive index (Figure 2). The variables analyzed by contrast enhancement ultrasonography were contrast wash-in time (s), time to peak enhancement (s), and washout time (s) (Figure 3); whole those by acoustic radiation force impulse elastography were qualitative deformability (deformable or not deformable) and mass mean shear wave velocity (m/s) (Figure 4).

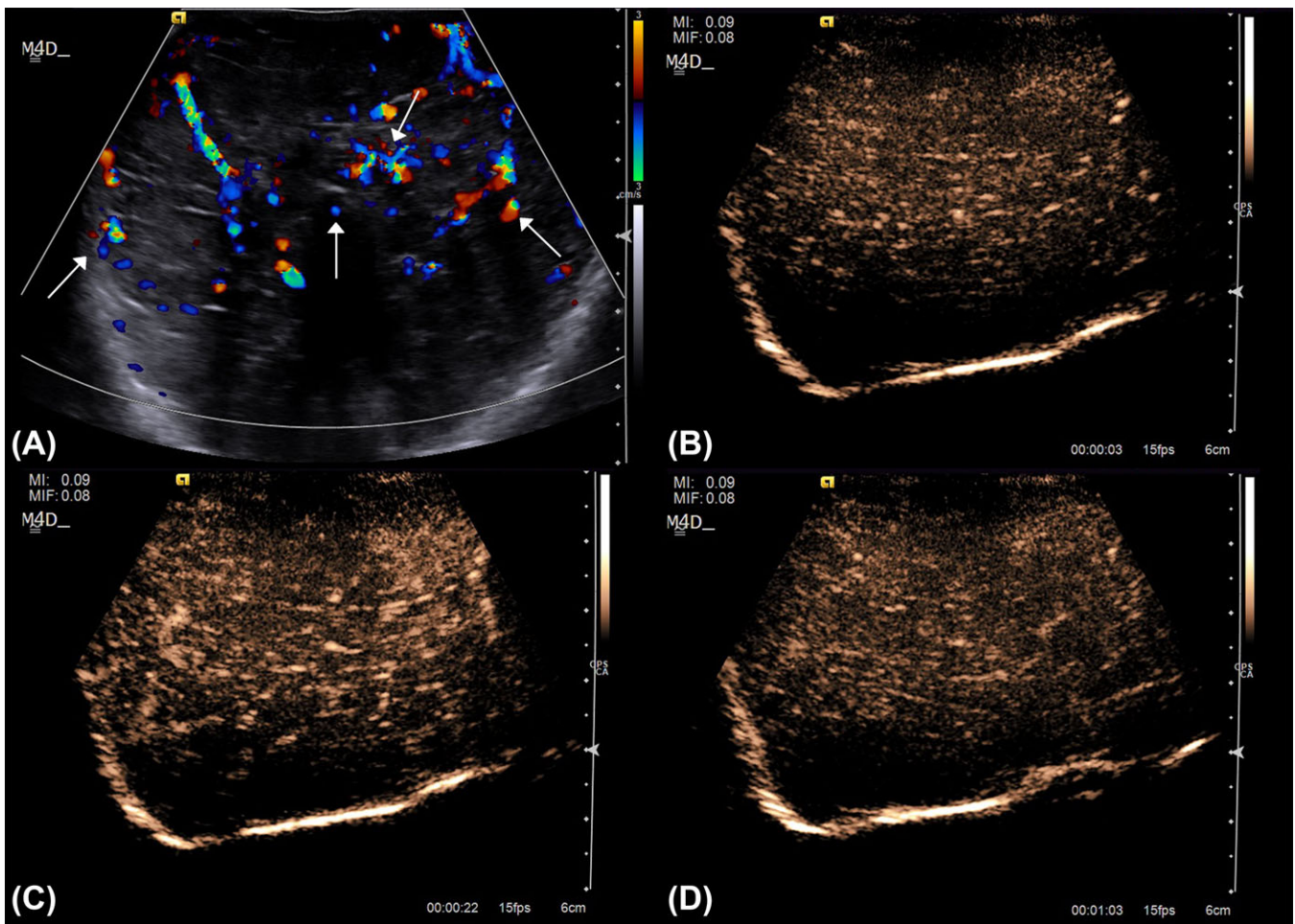


FIGURE 3 Contrast-enhanced ultrasonography image of spindle cell carcinoma in a female dog. A, Neovascularization (arrows) in mammary tumor by color Doppler ultrasonography. Contrast-enhanced ultrasonography: B, absence of contrast in tumor, C, peak enhancement, and D, washout phase [Color figure can be viewed at wileyonlinelibrary.com]

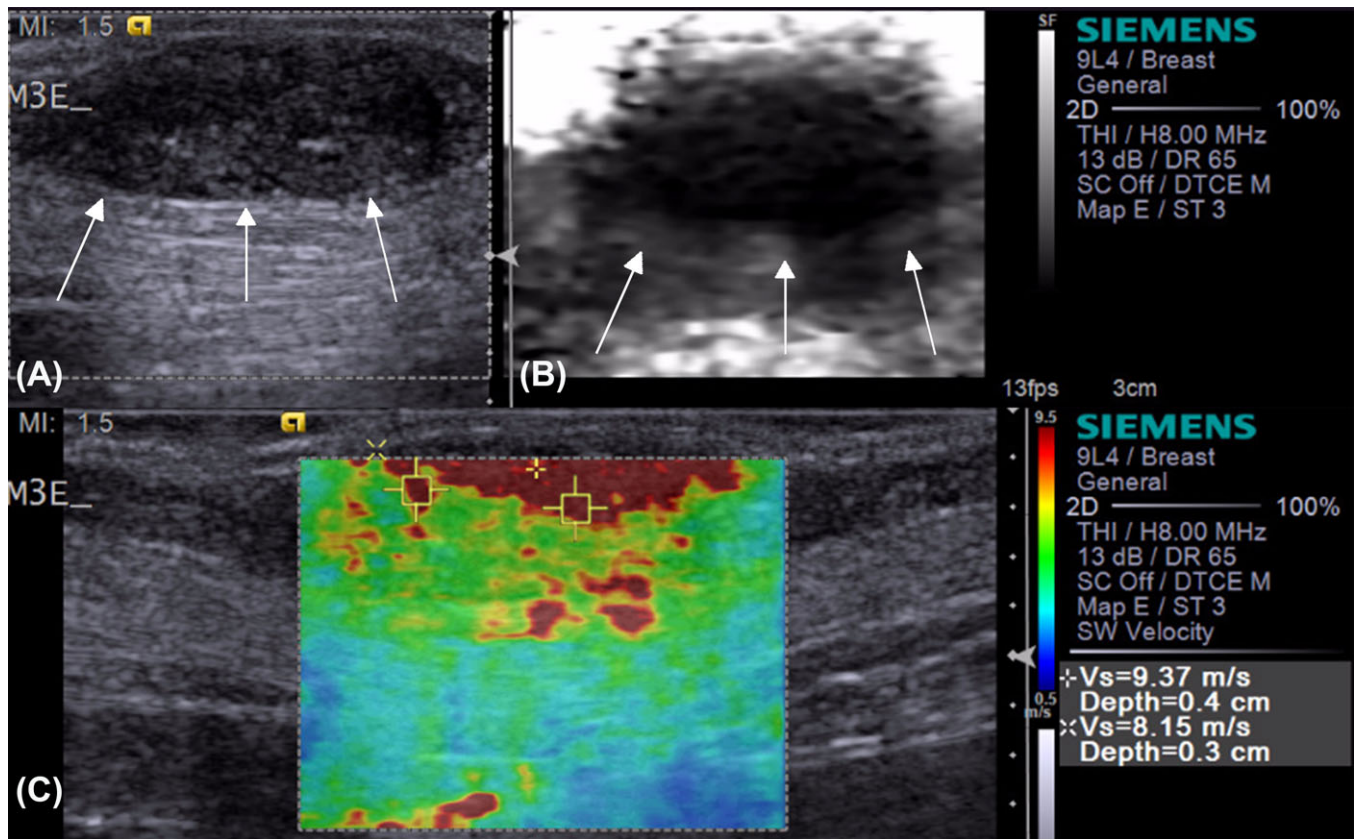


FIGURE 4 Acoustic radiation force impulse elastography image showing stiffness characteristics in malignant adenomyoepithelioma in a female dog. Image of the qualitative acoustic radiation force impulse: A, B-mode ultrasonography of mammary tumor (arrows) and B, elastogram revealing the characteristics of the stiffness of the lesion: not deformable and homogeneous with hard areas (black). C, Image of quantitative acoustic radiation force impulse in mammary tumor showing the presence of calipers in the parenchyma to record shear velocity [Color figure can be viewed at wileyonlinelibrary.com]

Histopathological diagnosis and classification for each of the carcinomas were based on interpretations by a single experienced pathologist (25 years) following the methodology previously described.⁷ Carcinomas were classified into simple, complex, or special types and grade I, II, or III according to literature.⁷⁻⁹

Statistical tests were selected and performed by one author (R.A.R.U.). Qualitative ultrasound variables were compared between carcinoma types and grades using Fisher's exact test. Quantitative variables were analyzed by analysis of variance once normal distribution of model residues and homoscedasticity of variances were confirmed by the Shapiro-Wilk and Bartlett tests, respectively. Cutoff point, sensitivity, specificity, accuracy, and area under curve were calculated for significantly ($P < 0.05$) different ultrasonography parameters, using histopathological classification as a reference for receiver-operating characteristic curve analysis in a logistic regression model. Statistical analysis was performed using R software version 3.3.0 (RTM Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

Of the 246 masses diagnosed as carcinomas (Table 1), 102 (42%) were classified as simple carcinomas, 136 (55%) as complex carcinomas,

and eight (3%) as special carcinomas. A total of 96 masses were classified as grade I (39%), 105 as grade II (43%), and 45 as grade III (18%) carcinomas.

The right mammary chain was affected in 114 cases (46%) and the left in 132 cases (54%); with seven (3%) carcinomas were located in the first, 38 (14%) in the second, 54 (22%) in the third, 72 (30%) in the fourth, and 75 (31%) in the fifth mammary glands. Owners reported that 204 (83%) neoplasms grew rapidly (in less than 6 months) and 42 (17%) grew slowly (in more than 6 months). Clinical examination revealed firm and soft consistency in 234 (95%) and 12 (5%) of tumors, respectively; with regular surface being observed in 157 tumors (64%) and irregular in 89 (36%).

The results obtained for the qualitative variables evaluated are summarized in Tables 2 and 3. The deformability evaluated by acoustic radiation force impulse-elastography was significantly different ($P = 0.0142$) between carcinoma types, with special carcinomas being proportionally less deformable than the rest. Thus, nondeformable acoustic radiation force impulse-elastography findings indicate, with 12% sensitivity, 100% specificity, and 75% accuracy, that mammary neoplasm to be a special type carcinoma. All other qualitative parameters did not vary between carcinoma types or grades ($P > 0.05$).

The quantitative results of B-mode, Doppler, contrast enhancement ultrasonography, and acoustic radiation force impulse-elastography

TABLE 1 Histopathological classifications and grades of the canine mammary carcinomas evaluated⁸

Carcinoma type	Diagnosis	Grade	Number
Simple	Tubular carcinoma	I	11
		II	17
		III	2
	Papillary carcinoma	I	5
		II	16
		III	6
	Solid carcinoma	I	3
		II	6
		III	9
	Lobular carcinoma in situ	II	8
	Lobular carcinoma in situ with microinvasion	II	3
	Micropapillary carcinoma	III	5
	Ductal carcinoma in situ	II	3
	Inflammatory carcinoma	II	3
	Squamous cell carcinoma	III	2
	Secretory carcinoma	II	2
	Anaplastic carcinoma	II	1
Complex	Carcinoma in a mixed tumor	I	77
		II	46
		III	6
	Complex carcinoma	III	7
Special	Malignant adenomyoepithelioma	III	6
	Spindle cell carcinoma	III	2

evaluation, in which carcinomas are classified by type and grade, are summarized in Tables 4 and 5. Only width:length ratio measured by B-mode ultrasonography and perfusion times by contrast enhancement ultrasonography were significantly ($P < 0.05$) different between carcinoma types or grades.

The width:length ratio was significantly higher ($P = 0.012$) in special type carcinomas, resulting in an identification cohort value greater than 0.53, with 80% sensitivity, 76% specificity, 78% accuracy, and 80% area under the curve.

Contrast wash-in and peak enhancement times were significantly higher ($P = 0.0205$ and 0.0361 , respectively) in simple and special type carcinomas. Therefore, cohort values lower than 7.5 s for wash-in times and lower than 13.5 s to peak enhancement were indicative of complex type carcinoma; with mean sensitivity of 62%, specificity of 60%, accuracy of 53%, and area under the curve of 62%. Similarly, contrast wash-in, peak enhancement, and washout times were significantly lower ($P = 0.0065$, 0.0109 , and 0.0242 ; respectively) in the grade I carcinomas. Thus, cohort values greater than 6.5 s for wash-in, 12.5 s for peak, and 64.5 s for washout times were indicatives of grades II and III carcinoma; with mean sensitivity of 68%, specificity of 62%, accuracy of 63%, and area under the curve of 66%.

4 | DISCUSSION

The mammary ultrasound exam by B-mode ultrasonography, contrast enhancement ultrasonography, and acoustic radiation force impulse-elastography enabled the identification of high-grade canine mammary carcinoma types with limited accuracy. Thus, these ultrasound techniques may assist in the presurgical identification of special and complex type carcinomas and grade II and III carcinomas with moderate efficacy.

Echotexture, margin, and invasiveness evaluation by B-mode ultrasonography did not allow differentiation of canine mammary carcinomas, probably due to the high variability in the morphostructural characteristics of each tumor type and consequently limiting the applicability of this method, in agreement with studies in women with breast carcinomas.^{11,12}

The large size of mammary masses has been described as a poor prognosis factor in female dogs, especially when associated with regional abnormalities of the lymphoid tissue, rapid growth, and/or ulceration.^{3,10,13,14} In literature, the use of B-mode ultrasonography to analyze 30 malignant, eight atypical, and 11 benign mammary lesions in female dogs concluded that the larger masses were correlated to high-grade malignancy and worse prognosis.¹⁵ However, in the present study, only width:length ratio greater than 0.53 obtained by B-mode ultrasonography enabled the identification of special type carcinomas with acceptable accuracy. These findings are in agreement with previous reports, which have identified this ratio to be an indicator of malignancy in mammary tumors in women and female dogs.^{6,7,16} Thus, this parameter can be used as a simple and economical tool in the diagnosis of mammary neoplasms in female dogs.

Color and spectral Doppler variables in this study did not provide any data that could be used in the identification of high-grade mammary carcinomas. All the tumors evaluated showed signs of neovascularization and high vascular indexes, both of which are reported indicators of malignancy.^{2,7,17} Although it was expected that the high-grade carcinomas would induce greater vascular development to ensure their growth, these changes were neither detected by Doppler ultrasonography nor were the tissue alterations that occurred within these tumors, such as central necrosis, which enables aggressive behavior without the need for an exacerbated blood supply.^{11,18}

Contrast-enhanced ultrasonography, which enables the noninvasive evaluation of tissue perfusion, identified complex carcinomas with moderate accuracy, due to the shorter periods of contrast wash-in and peak enhancement times observed in this type of tumor. These findings corroborated previous reports, which correlated lower perfusion times with more severe types of breast tumors in women.^{19,20} On the other hand, an increase in perfusion times (wash-in, peak enhancement, and washout times) enabled the detection of grade II and III carcinomas, with moderate accuracy. These results contradicted reports on breast cancer in women.^{19,20} However, it is possible that tumors with complex behaviors expand differently in the female dog due to the anatomical characteristics and larger mammary glands when compared to women.^{8,21} Furthermore, this different configuration could possibly lead to the slower perfusion observed in high-grade carcinomas.

TABLE 2 Qualitative parameters of canine mammary carcinomas according to B-mode and Doppler ultrasonography, and acoustic radiation force impulse elastography

Parameter		Findings	Carcinoma type			P-value
			Simple (n = 102)	Complex (n = 136)	Special (n = 8)	
B-Mode ultrasonography						
Echotexture		Homogenous (%)	30	32	13	0.6002
		Heterogeneous (%)	70	68	88	
Contours or margins		Defined (%)	98	100	87	0.1950
		Undefined (%)	2	0	13	
Invasiveness		Present (%)	0	0	0	1.000
		Absent (%)	100	100	100	
Doppler ultrasonography						
Vascularization		Present (%)	100	100	100	1.000
		Absent (%)	0	0	0	
ARFI elastography						
Deformability		Deformable (%)	18	31	0	0.0142*
		Not deformable (%)	82	69	100	

Notes. ARFI, acoustic radiation force impulse.

*Significantly different by the Fisher exact test ($P < 0.05$).

TABLE 3 Qualitative parameters of canine mammary carcinomas according to B-mode and Doppler ultrasonography, and acoustic radiation force impulse elastography

		Carcinoma grade			P-value
		I (n = 96)	II (n = 105)	III (n = 45)	
B-Mode ultrasonography					
Echotexture	Homogenous (%)	34	32	20	0.2117
	Heterogeneous (%)	66	68	80	
Contours or margins	Defined (%)	100	100	98	0.1829
	Undefined (%)	0	0	2	
Invasiveness	Present (%)	0	0	0	1.0000
	Absent (%)	100	100	100	
Doppler ultrasonography					
Vascularization	Present (%)	100	100	100	1.0000
	Absent (%)	0	0	0	
ARFI elastography					
Deformability	Deformable (%)	26	29	11	0.0659
	Not deformable (%)	74	71	89	

Notes. ARFI, acoustic radiation force impulse.

The use of acoustic radiation force impulse–elastography enables qualitative (deformable or nondeformable) and quantitative (shear wave velocity) evaluation. The qualitative evaluation in this study revealed that 84% of carcinomas had nondeformable characteristics, in agreement with previous studies that have considered this as malignancy factor.⁷ Although 100% of the special carcinomas evaluated were classified as nondeformable at 100% specificity, the sensitivity of 12% does not enable this parameter to be considered a test with diagnostic value in the identification of special carcinomas. Contrary to expectations, quantitative evaluation did not result in significant differences between carcinoma types or grades. Thus, the carcinoma masses analyzed presented high velocity (6.0 ± 2.4 m/s) correlated

with low elasticity and rigid consistency, as previously described.^{7,22,23} Even though special and complex carcinomas showed greater rigidity in the qualitative and quantitative evaluation, the diagnostic technique is not sensitive enough for differentiation of these tumor types. The fast development of increasingly accurate elastographic techniques may enable this differentiation in the near future.

The current study has some limitations that must be addressed. Although the sample size was adequate ($n = 246$), the prevalence of special carcinomas was very low ($n = 6$, 3.3%), which could have compromised the sensitivity of the techniques analyzed in the identification of this type of carcinoma. Mean tumoral size was small ($(2.6 \pm 1.7) \times (1.6 \pm 1.2)$ cm), which may have made ultrasonography

TABLE 4 Mean $\pm$ standard deviation of quantitative variables of canine mammary carcinomas according to B-mode and Doppler ultrasonography, contrast enhanced ultrasonography, and acoustic radiation force impulse elastography

	Carcinoma type			
Variables	Simple (n = 102)	Complex (n = 136)	Special (n = 8)	P-value
B-Mode ultrasonography				
Longitudinal width (cm)	2.52 ± 1.60	2.41 ± 1.64	3.58 ± 2.19	0.3790
Longitudinal length (cm)	1.12 ± 0.94	1.19 ± 1.14	2.09 ± 1.35	0.2892
Width:length ratio	0.43 ± 0.14 ^a	0.47 ± 0.15 ^a	0.56 ± 0.07 ^b	0.0117*
Transverse height (cm)	1.82 ± 1.30	2.04 ± 1.67	3.17 ± 1.65	0.1522
Transverse width (cm)	1.42 ± 1.20	1.41 ± 1.25	2.06 ± 1.30	0.4890
Width:height ratio	0.48 ± 0.28	0.90 ± 0.81	0.60 ± 0.15	0.9034
Volume (cm ³)	10.23 ± 24.46	14.40 ± 38.99	28.70 ± 26.40	0.3750
Doppler ultrasonography				
Systolic velocity (cm/s)	38.02 ± 26.30	34.45 ± 24.44	68.30 ± 45.30	0.2259
Diastolic velocity (cm/s)	8.78 ± 6.01	7.67 ± 6.00	24.90 ± 30.80	0.1088
Resistive index	0.75 ± 0.10	0.77 ± 0.10	0.74 ± 0.19	0.3903
Contrast-enhanced ultrasonography				
Wash-in time (s)	9.64 ± 4.79 ^a	8.07 ± 4.99 ^b	11.88 ± 5.08 ^a	0.0205*
Time to peak (s)	15.25 ± 5.69 ^a	13.79 ± 5.60 ^b	18.25 ± 5.01 ^a	0.0361*
Wash-out time (s)	62.42 ± 21.88	64.38 ± 22.59	76.25 ± 25.87	0.2739
ARFI elastography				
SWV (m/s)	5.53 ± 2.23	5.88 ± 2.57	6.59 ± 2.46	0.3163

Notes. Different letters (a and b) on the same row indicate significant difference between carcinoma types by the Tukey test ($P < 0.05$). ARFI, acoustic radiation force impulse; CEUS, contrast-enhanced ultrasonography; SD, standard deviation; SWV, shear wave velocity.

*Significant difference by analysis of variance ($P < 0.05$).

TABLE 5 Mean $\pm$ standard deviation of quantitative variables of canine mammary carcinomas according to B-mode and Doppler ultrasonography, contrast-enhanced ultrasonography, and acoustic radiation force impulse elastography

		Carcinoma grade			
Variables		I (n = 96)	II (n = 105)	III (n = 45)	P-value
B-Mode ultrasonography					
Longitudinal width (cm)		1.04 ± 0.94	1.17 ± 1.08	1.56 ± 1.25	0.2595
Longitudinal length (cm)		1.04 ± 0.94	1.17 ± 1.08	1.56 ± 1.25	0.1219
Width:length ratio		0.44 ± 0.15	0.45 ± 0.14	0.50 ± 0.15	0.0940
Transverse height (cm)		2.00 ± 1.70	1.83 ± 1.34	2.29 ± 1.57	0.4798
Transverse width (cm)		1.30 ± 1.04	1.44 ± 1.36	1.70 ± 1.29	0.2646
Width:height ratio		0.96 ± 0.92	0.97 ± 0.94	0.95 ± 0.93	0.6242
Volume (cm ³)		9.63 ± 28.78	13.59 ± 37.66	19.47 ± 32.50	0.2475
Doppler ultrasonography					
Systolic velocity (cm/s)		36.45 ± 29.34	33.81 ± 19.20	46.44 ± 33.69	0.2356
Diastolic velocity (cm/s)		7.63 ± 5.89	8.21 ± 6.38	12.53 ± 15.38	0.1163
Resistive index		0.77 ± 0.10	0.76 ± 0.10	0.74 ± 0.12	0.3903
Contrast-enhanced ultrasonography					
Wash-in time (s)		7.50 ± 4.21 ^a	10.12 ± 5.34 ^b	9.85 ± 4.82 ^b	0.0065*
Time to peak (s)		13.13 ± 5.16 ^a	15.40 ± 5.95 ^b	16.62 ± 5.38 ^b	0.0109*
Washout time (s)		61.52 ± 19.62 ^a	71.31 ± 22.59 ^b	75.00 ± 24.67 ^b	0.0242*
ARFI elastography					
SWV (m/s)		6.06 ± 2.49	5.51 ± 2.40	5.69 ± 2.38	0.3497

Notes. Different letters (a and b) on the same row indicate significant difference between carcinoma types by the Tukey test ($P < 0.05$). ARFI, acoustic radiation force impulse; CEUS, contrast-enhanced ultrasonography; SD, standard deviation; SWV, shear wave velocity.

*Significant difference by Analysis of variance ($P < 0.05$).

evaluation difficult. Lastly, contrast enhancement ultrasonography and acoustic radiation force impulse elastography techniques are currently only available at research centers and university hospitals, temporarily limiting the clinical application of these exams.

In conclusion, mammary ultrasonography evaluation by B-mode, contrast enhancement ultrasonography, and acoustic radiation force impulse–elastography enabled the grade and some characteristics of high-grade mammary carcinomas in this sample of female dogs to be identified with limited accuracy. A width:length ratio greater than 0.53 enabled the identification of special type carcinomas with 80% sensitivity and 76% specificity. Contrast wash-in and peak enhancement times lower than 7.5 and 13.5 s, respectively, were indicative of a complex type carcinoma with 62% sensitivity and 60% specificity. Contrast wash-in, peak enhancement, and washout times greater than 6.5, 12.5, and 64.5 s, respectively; were indicative of grade II and III carcinoma with 68% sensitivity and 62% specificity. This study provides relevant information that may contribute to the research and clinical management of oncological canine patients.

LIST OF AUTHOR CONTRIBUTIONS

Category 1

- (a) Conception and Design: Feliciano MAR, Ramirez RAU, Carvalho CF
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Category 2

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