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PAPER

Contrast-enhanced ultrasonography features of hepatobiliary neoplasms in cats

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Abstract

Background Contrast-enhanced ultrasonography (CEUS) features of primary hepatobiliary neoplasms have been reported in dogs but no information is available in cats.

Methods Qualitative and quantitative features of bile duct adenomas (BDAs, n=20), bile duct carcinomas (BDCs, n=16), and hepatocellular carcinomas (HCCs, n=8) are described in 44 cats.

Results There was an overlap in CEUS qualitative features between different histotypes, both in wash-in and wash-out phases. Distinction between different neoplasms based only on the CEUS qualitative features was not possible. At peak of enhancement, the BDAs, BDCs and HCCs showed a large range of echogenicities, from hypoenhancement to hyperenhancement, in comparison to the liver parenchyma. Eight of 20 BDAs showed inhomogeneous hyperenhancement during wash-in, which is a feature reported as typical of malignant lesions in dogs. BDC had a significantly faster wash-in compared with both BDA and HCC but the diagnostic accuracy of all the included quantitative variables was only moderate. No significant differences in the wash-out quantitative features of BDA and BDC were evident.

Conclusion There is poor evidence that CEUS may be used to distinguish between different primary hepatobiliary neoplasms in cats.

Introduction

Primary hepatobiliary neoplasms in cats account for 1 per cent to 2.9 per cent of the total neoplasms in this species, with the most common histotype being bile duct adenoma (BDA) (45.4 per cent), followed by bile duct carcinoma (BDC) (36.3 per cent) and hepatocellular carcinoma (HCC) (18.1 per cent); other primary hepatic neoplasms in cats include sarcomas and neuroendocrine tumours but these are much rarer.[1] B-mode ultrasonographic features of focal liver disease are reported to be non-specific, and additional invasive procedures, such as cytology or histopathology, are currently required to determine the histotype of the mass or nodule under examination.[2]

Possible applications of contrast-enhanced ultrasonography (CEUS) in dogs range from the characterisation of liver masses,[3–5] to the distinction between hepatitis and other non-inflammatory disorders,[6] to the detection of urinary bladder transitional carcinoma,[7] to the identification of high-grade mammary carcinomas,[8] to the characterisation of adrenal tumours[9] and gall bladder disease,[10] to the description of pancreatic acute inflammation,[11] tumours[12] and insulinoma.[4] The available literature reporting CEUS use in cats is limited and focuses mainly on the evaluation of kidney perfusion[13] and on the distinction of neoplastic from non-neoplastic intrathoracic masses.[14] Furthermore, there is one study in cats and dogs describing renal lesions on CEUS.[15]

Normal CEUS features of the feline liver have been reported,[16] but no references describing the CEUS findings for hepatic nodular lesions in cats are available. Therefore, the primary aim of the present study is to describe the CEUS features of primary hepatobiliary neoplasms in cats. Furthermore, as only the distinction between tumours arising in the liver parenchyma from

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Table 1 Number of cases showing qualitative features at B-mode ultrasound: cytopathology classification

	Echogenicity				Aspect		Diffusion	
	Isoechoic	Hypoechoic	Hyperechoic	Mixed	Solid	Cystic	Focal	Diffuse
Cytological diagnosis								
BDA	0	0	16	4	10	10	20	0
BDC	0	5	4	7	10	6	14	2
HCC	0	0	0	8	4	4	3	5
P value	<0.001				0.725		<0.001	

BDA, bile duct adenoma; BDC, bile duct carcinoma; HCC, hepatocellular carcinoma.

those originating in the bile ducts (but not between BDA and BDC) is possible[17] by means of cytology, the secondary aim of this study was to evaluate the diagnostic accuracy of CEUS in the distinction between BDA and BDC.

Methods

Study population and inclusion criteria

Cats referred to Ultravet (Ultravet, Via E. Fermi 59, San Giovanni in Persiceto, Bologna) and Tyrus Veterinary Clinic (Tyrus Veterinary Clinic, Via A. Bartocci 1/G, Terni, Italy) between January 2010 and January 2019 for specialty liver CEUS examination, and having a single cytologically diagnosed liver mass, were included in this study. Complete signalment was recorded for each patient.

Different inclusion criteria were adopted for benign and malignant lesions because cytology is reported to have a low sensitivity but a very high positive predictive value (PPV) for neoplasms.[18] Indeed, only cats having a single cytological diagnosis of malignant neoplasm or two consecutive cytological diagnoses of benign neoplasm performed within an interval of at least six months were included. On the contrary, those animals: 1) with single cytological diagnosis of benign lesion; 2) with cytological diagnosis of liver or diffuse metastases; 3) having received chemotherapy before the examination; and 4) with another cytological diagnosis than BDA, BDC and HCC, were excluded from the study. These inclusion criteria were adopted because the distinction between BDA and BDC is reported to be challenging through cytology.

The results of additional tests performed in the present facilities (blood examination, cytology of other organs) were also collected. All the procedures were carried out in accordance with the relevant guidelines and regulations.

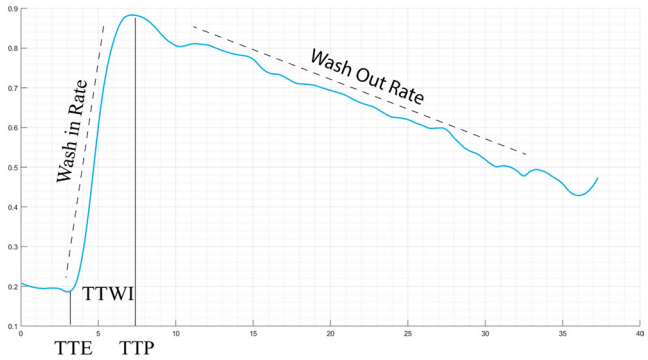

Figure 1 Example of a time-intensity curve explaining how the wash-in rate, the wash-out rate, the time to enhancement (TTE), the time to peak (TTP) and the time to wash-in (TTWI) were calculated.

Analysis of the B-mode examinations

The B-mode ultrasonographic examinations were performed by two veterinarians (GR and PB) using three different ultrasonographic scanners, GE Logiq E9 (GE Medical Systems), Esaote MyLab70 Gold (Esaote Italia) or Esaote Twice (Esaote Italia). Linear probes (9–3 Mhz) were always used. The gain and time-gain compensation were adjusted to optimise the image during the examination. The following B-mode qualitative features were evaluated for each lesion: 1) echogenicity in comparison to the surrounding liver parenchyma, classified as hypoechoic, hyperechoic, isoechoic or having a mixed echogenicity; 2) aspect classified as solid or cystic; 3) distribution classified as focal or diffuse (the lesion involved more than one liver lobe and the distinction between the lesion and the normal liver parenchyma was unclear).

Analysis of the CEUS examinations

All the examinations were performed by two veterinarians (GR and PB) following the same protocol: 1) eight-hour fasting period was observed before each CEUS examination; 2) the mechanical index was set to a very low value (0.02); 3) Sonovue was administered intravenously at the dose of 0.05 ml/kg; and 4) the lesions were scanned for at least one minute or until the end of the wash-out phase.

All the CEUS examinations were reviewed by the same operators (TB, GR and SB). As the original digital imaging and communications in Medicine (DICOM) videos were no longer available, time-intensity curves were generated from the .avi files using a purpose-built MATLAB (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States.) script. One region of interest (ROI) was manually placed by one operator (SB) on the lesion. In case of inhomogeneous enhancement the ROI was placed in the contrast-enhancing portions of the mass. The ROI was placed so that only the lesion (or a portion thereof) was included in the analysis. The following quantitative parameters were calculated from the time-intensity curves: 1) time from injection to enhancement (TTE); 2) time from injection to maximum intensity, or

Table 2 Contrast-enhancement quantitative features calculated from the graphs. Data are reported as means with the limits of the overall range

	TTE	TTP	TTWI	WI-rate	WO-rate
Cytological diagnosis					
BDA	6.900 (4 to 10)	18.900 (9 to 34)	12.000 (2 to 27)	0.129 (0.012 to 0.307)	−0.034 (−0.160 to −0.005)
BDC	4.688 (1 to 9)	12.937 (4 to 28)	8.250 (3 to 23)	0.337 (0.054 to 1.544)	−0.038 (−0.276 to −0.007)
HCC	5.625 (4 to 7)	16.875 (9 to 34)	11.250 (4 to 28)	0.168 (0.023 to 0.538)	−0.024 (−0.066 to −0.005)
P value	0.004	0.033	0.173	0.036	0.827

BDA, bile duct adenoma; BDC, bile duct carcinoma; HCC, hepatocellular carcinoma; TTE, time from injection to enhancement; TTP, time to peak; TTWI, time to wash-in; WI, wash-in; WO, wash-out.

time to peak (TTP); 3) time to wash-in (TTWI), expressed as the difference between TTE and TTP; 4) wash-in rate, calculated as the slope of the time-intensity curve during wash-in; and 5) wash-out rate, calculated as the slope of the time-intensity curve during wash-out. The qualitative parameters—namely contrast-enhancement features—were evaluated both during the wash-in phase and the wash-out phase. The following contrast-enhancement qualitative features: 1) enhancement degree compared with the ultrasonographically normal liver parenchyma (isoenhancing, hypoenhancing or hyperenhancing); 2) homogeneity (homogeneous or inhomogeneous); and 3) pattern of contrast enhancement (centripetal, centrifugal or diffuse), were evaluated for each lesion during both wash-in (from TTE to TTP) and wash-out (from 15 seconds after peak of enhancement determined by the time-intensity curves). The distribution of the contrast medium (central, peripheral or diffuse) was evaluated only at TTP during wash-in; the margins of the lesions (clear or feathered) were evaluated only during wash-out.

Cytopathological examinations

Cases were classified according to cytological analysis. Lesions were classified as primary hepatic tumours when the hepatocytes showed marked signs of cellular atypia. Lesions were classified as biliary tumours when the predominant population in the cytological slides were small sheets or clusters of bile duct epithelial cells arranged in tubular or acinar cytoarchitectures, showing no or few atypia criteria, and with a minimal amount of relatively clear cytoplasm.[17]

Statistical evaluation of the B-mode and CEUS features of primary hepatobiliary neoplasms in cats

The statistical evaluation was performed using the MedCalc Statistical Software V.15. Differences in the distribution of qualitative B-mode and CEUS parameters among the different groups were tested with the chi-squared (x^2) test or with Fisher's exact method.[19] The differences in distribution of the quantitative parameters in relation to the different groups were analysed using one-way analysis of variance (ANOVA) for normally distributed data or with the Kruskal-Wallis test for non-normally distributed data. The Tukey-Kramer method was used for multiple comparison tests. A value pf P less than 0.05 was considered as statistically significant for each test. Power analysis was performed with a post hoc test on the parameter showing the best statistical results.

The diagnostic accuracy of CEUS in the distinction between BDA and BDC was assessed. The cut-off points, sensitivity, specificity, PPV, negative predictive value (NPV) and the area under the curve (AUC) of quantitative variables showing statistical significance were analysed using the receiver operator characteristics ROC curves. The AUC value as a criterion of discrimination accuracy was classified as low (0.5–0.7), moderate (0.7–0.9) or high (>0.9).[20]

Results

Study population

Research in the archive retrieved 51 cats in total. Twenty-two were diagnosed with BDA, 16 with BDC,

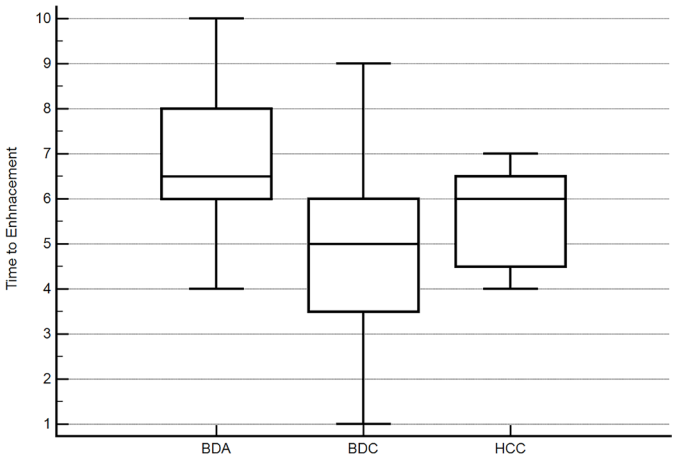

Figure 2 Box and whisker plot of the time to enhancement for bile duct adenoma (BDA), bile duct carcinoma (BDC) and hepatocellular carcinoma (HCC).

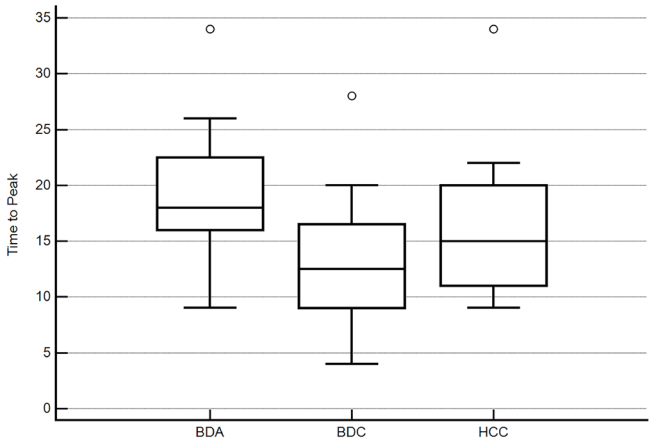

Figure 3 Box and whisker plot of the time to peak for bile duct adenoma (BDA), bile duct carcinoma (BDC) and hepatocellular carcinoma (HCC).

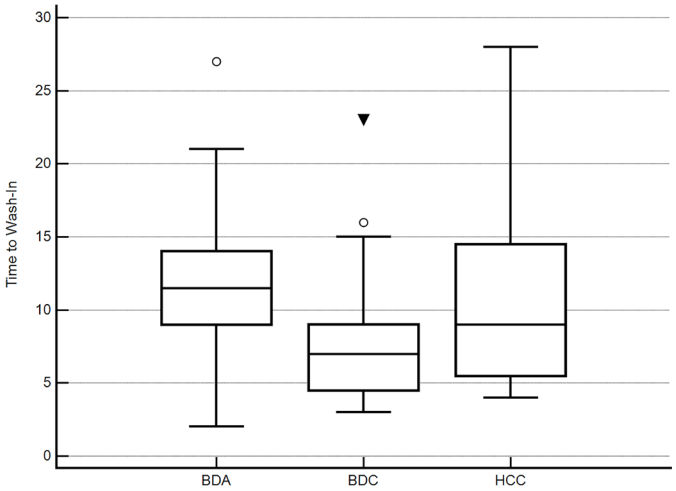

Figure 4 Box and whisker plot of the time to wash-in for bile duct adenoma (BDA), bile duct carcinoma (BDC) and hepatocellular carcinoma (HCC).

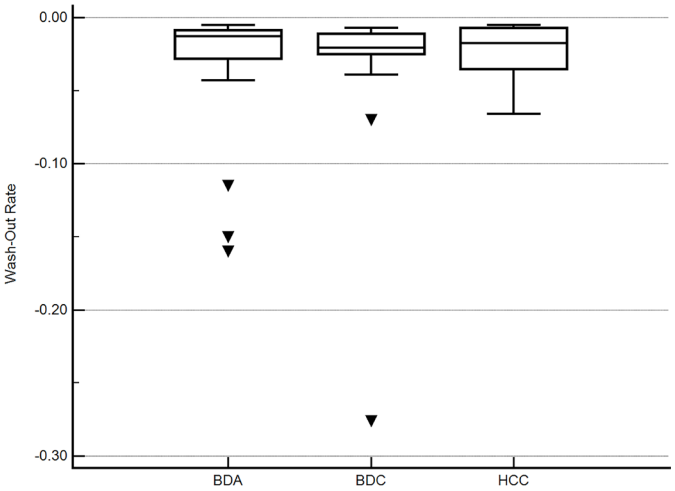

Figure 6 Box and whisker plot of the wash-out rate for bile duct adenoma (BDA), bile duct carcinoma (BDC) and hepatocellular carcinoma (HCC).

8 with HCC, 3 with lymphoma and 2 with metastasis from pancreatic carcinoma. No cats were diagnosed with nodular hyperplasia or hepatic adenoma. Two of the 22 cats diagnosed with BDA had only a single cytological examination and therefore were discarded from the study. Likewise, the three cats diagnosed with lymphoma and the two cats diagnosed with metastasis from pancreatic cancer were not included in the analysis. Therefore, 44 cats in total were included in the present study. Cytologically confirmed metastases to the spleen were evident only in four cats with HCC.

Analysis of the B-mode ultrasonographic examination

Summary statistics of B-mode ultrasonographic qualitative features of liver masses with cytopathological classification are reported in table 1. Echogenicity of the non-cystic, and solid components of the hepatobiliary lesions was significantly different (x^2=27.581; p<0.001) among the cytopathological groups. Indeed, all the HCCs had a mixed echogenicity, BDAs were mainly hyperechoic (16/20) and BDC showed almost all the possible echogenicities (except isoechoic). A statistically significant difference was found also for the distribution of the lesion (x^2=16.903; p<0.001). Indeed, BDAs were always focal, BDCs were mainly focal (14/16), whereas HCCs showed similar proportions of focal (3/8) and diffuse (5/8) lesions. On the contrary, no statistically significant differences were evident in the aspect (solid or cystic) of the different cytopathological groups (x^2=0.642; p=0.715).

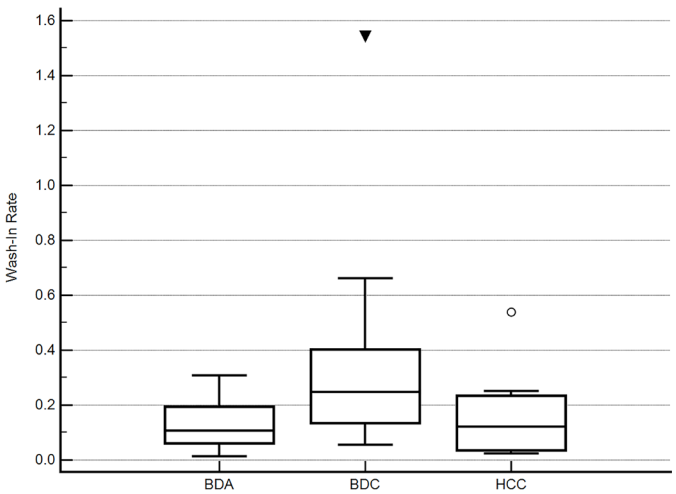

Figure 5 Box and whisker plot of the wash-in rate for bile duct adenoma (BDA), bile duct carcinoma (BDC) and hepatocellular carcinoma (HCC).

Analysis of the CEUS examination

A graph illustrating how the quantitative parameters were calculated is reported in figure 1. Summary statistics of the quantitative parameters for the different groups are reported in table 2. Multiple comparison graphs of TTE, TTP, TTWI, wash-in rate and wash-out rate are reported in figures 2–6, respectively. TTP, TTE and TTWI were normally distributed and therefore the differences between groups were calculated by means of ANOVA. The wash-in rate and the wash-out rate were not normally distributed and therefore the differences between groups were calculated using the Kruskal-Wallis test. Statistically significant differences between groups were evident in the TTE (F=6.313; p=0.004) with BDC displaying, on average, a faster enhancement compared with BDA. Likewise, TTP was significantly faster (F=3.724; p=0.033) in BDC compared with BDA. As a consequence, BDC displayed a significantly faster (x^2=8.296; p=0.016) wash-in rate compared with BDA. No differences were evident between HCC and both BDA and BDC for all the above parameters. No statistically significant differences were evident in TTWI (F=1.148; p=0.238) or in the wash-out rate (x^2=1.067; p=0.585).

Power analysis was performed on TTE. Effect size was calculated from the means of individual groups with an overall SD of 2.09 seconds. A moderate effect size (r^2=0.481) was evident; given an α probability error of 0.05, the power of the test was high (1 – β error=0.812).

A summary of the CEUS qualitative features evaluated during wash-in in relation to the results of the cytological examination is reported in table 3. A summary of the CEUS qualitative features evaluated during wash-out in relation to the result of the cytological analysis is reported in table 4. Statistically significant differences

Table 3 Number of cases showing qualitative contrast-enhancement feature during wash-in: cytophathology classification

	Enhancement degree			Homogeneity		Pattern			Distribution		
	Isoenhancing	Hypoenhancing	Hyperenhancing	Homogeneous	Inhomogeneous	Centripetal	Centrifugal	Diffuse	Central	Peripheral	Diffuse
Cytological diagnosis											
BDA	10	2	8	8	12	0	0	20	3	2	15
BDC	3	3	10	3	13	0	1	15	0	4	12
HCC	1	0	7	0	8	3	0	5	1	3	4
P value	0.092			0.067		0.003			0.260		

BDA, bile duct adenoma; BDC, bile duct carcinoma; HCC, hepatocellular carcinoma.

Table 4 Number of cases showing qualitative contrast-enhancement feature during wash-out: cytophathology classification

	Enhancement degree			Homogeneity		Pattern			Margins	
	Isoenhancing	Hypoenhancing	Hyperenhancing	Homogeneous	Inhomogeneous	Centripetal	Centrifugal	Diffuse	Clear	Feathered
Cytological diagnosis										
BDA	0	19	1	4	16	0	8	12	13	7
BDC	0	15	1	2	14	0	6	10	10	6
HCC	0	7	1	2	6	0	6	2	2	6
P value	0.772			0.725		0.177			0.131	

BDA, bile duct adenoma; BDC, bile duct carcinoma; HCC, hepatocellular carcinoma.

in the qualitative parameters evaluated during wash-in were evident only in the pattern of contrast medium enhancement (x^2=16.156; p=0.003). Distribution (x^2=5.272; p=0.264), enhancement degree (x^2=7.997; p=0.092) and homogeneity (x^2=5.40; p=0.067) of the lesions showed no significant differences. Likewise, none of the wash-out qualitative features showed statistically significant differences: enhancement degree (x^2=0.519; p=0.772), homogeneity (x^2=0.642; p=0.725), pattern (x^2=3.465; p=0.177), margins (x^2=4.057; p=0.131). CEUS images of a BDA, a BDC and an HCC are illustrated in figures 7–9, respectively.

Diagnostic accuracy in the distinction between BDA and BDC

Only TTE, TTP and wash-in rate were included in the analysis. The diagnostic accuracy of all the variables was classified as moderate. The cut-off points, sensitivity, specificity, PPV, NPV and AUC are reported in table 5 and figure 10.

Discussion

The results of the present study reveal that BDAs, BDCs and HCCs have variable qualitative B-mode and CEUS features in cats. Both the distribution and the echogenicity of the lesions in B-mode showed significant differences. On the other hand, the large overlap of the B-mode features does not allow the distinction among the included cytopathological groups based only on this imaging modality. Likewise, the pattern of contrast enhancement was the only CEUS qualitative parameter showing statistically significant difference but, as reported in table 2, most of the lesions (40 out of 44) showed a diffuse contrast-enhancement pattern, and, as such, this parameter too was not useful in a clinical setting.

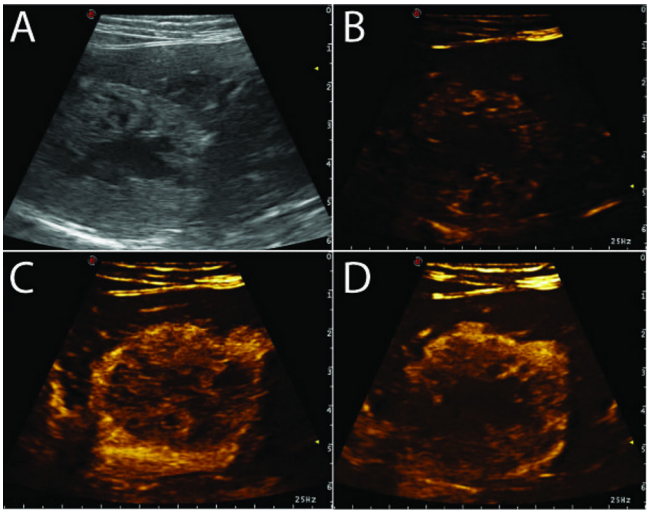

Figure 7 Contrast-enhanced ultrasonographic (CEUS) images of a bile duct adenoma (BDA) showing inhomogeneous hyperenhancing wash-in and inhomogeneous wash-out. (A) B-mode ultrasonographic image of the lesion. (B,C,D) CEUS images of the lesion 10 seconds, 20 seconds and 45 seconds after contrast medium injection, respectively.

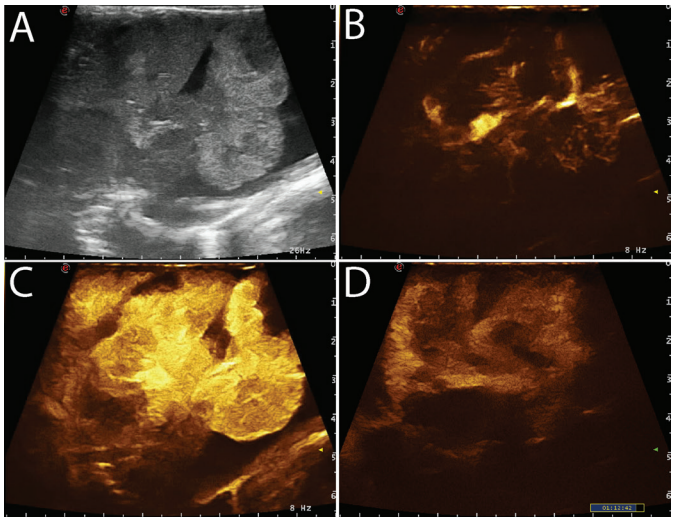

Figure 8 Contrast-enhanced ultrasonographic (CEUS) images of a bile duct carcinoma (BDC) showing inhomogeneous hyperenhancing wash-in and inhomogeneous wash-out. (A) B-mode ultrasonographic image of the lesion. (B,C,D) CEUS images of the lesion 4 seconds, 9 seconds and 80 seconds after contrast medium injection, respectively.

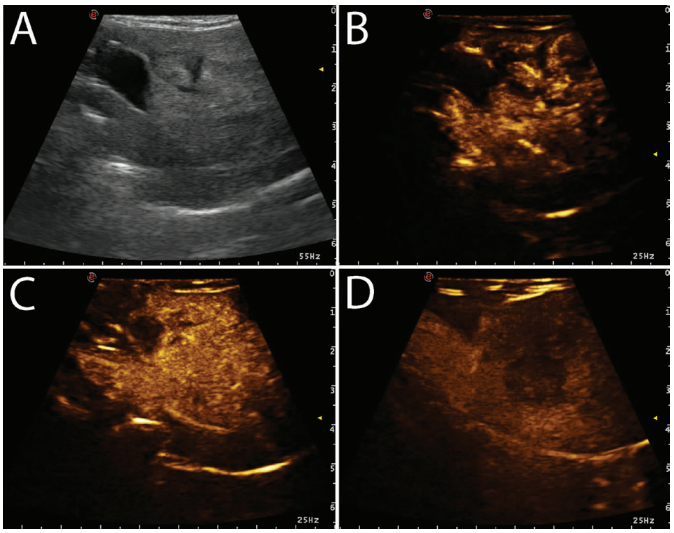

Figure 9 Contrast-enhanced ultrasonographic (CEUS) images of a hepatocellular carcinoma (HCC) showing homogeneous isoenhancing wash-in and inhomogeneous wash-out. (A) B-mode ultrasonographic image of the lesion. (B,C,D) CEUS images of the lesion 8 seconds, 14 seconds and 71 seconds after contrast medium injection, respectively.

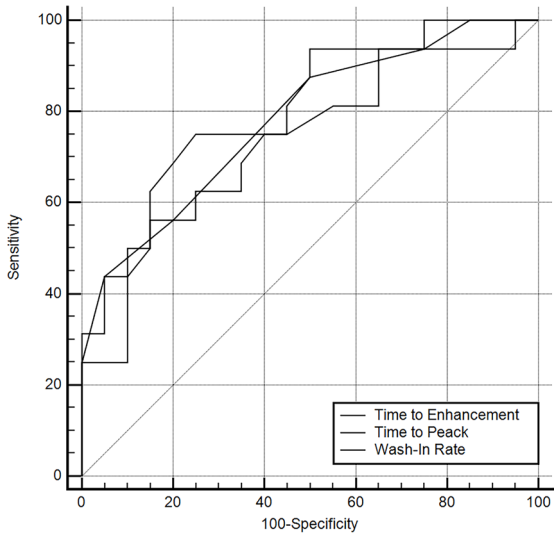

Figure 10 Receiver operator characteristics curve of the wash-in rate, time to wash-in, time to enhancement in the distinction between bile duct adenomas and bile duct carcinomas.

There was a large overlap in the CEUS qualitative features of BDA and BDC, during both the wash-in and wash-out phases; hence, the distinction between these two pathologies based only on the CEUS qualitative features is not possible. In particular, both BDA and BDC showed very variable wash-in (table 2) and wash-out (table 3) features. Furthermore, 8/20 BDAs showed inhomogeneous hyperenhancement during wash-in, a feature that is reported to be typical of malignant lesions both in dogs[5] and in human beings.[21] Interestingly, almost all the BDAs (19/20) and BDCs (15/16) were hypoenhancing during wash-out. BDA is uncommon both in dogs and in human beings, and, to the best of the authors' knowledge, the CEUS characteristics of BDA have been reported only in one dog.[22] Interestingly, the CEUS characteristics of BDA reported in this dog (hyperenhancement during wash-in and hypoenhancement during wash-out) were similar to those described here. Other ultrasonographic findings, such as lesion distribution (localised v diffuse), the presence or absence of metastasis to the local (hepatic) or distant lymph nodes, or metastasis to other organs (heart, lungs, etc), might be useful in the distinction between BDA and BDC.[1]

By contrast, statistical analysis of quantitative parameters revealed that BDA had a slower TTE and a slower TTP, thus resulting in a lower wash-in rate than BDC (table 4). While CEUS is reported to have a very high diagnostic accuracy (with a specificity of 100 per cent and a PPV of 94.1 per cent) in the discrimination between benign and malignant lesions in dogs,[5] only a moderate diagnostic accuracy in the distinction between BDA and BDC emerges from this research in cats. Such a moderate diagnostic accuracy for all the quantitative parameters is most likely related to the intrinsic nature of BDA. Indeed, BDAs are mostly cystic formations that, at least in human beings, are thought to arise from an inflammatory condition,[23] and therefore have an intense arterial supply and a relative lack of portal vessels. Indeed BDAs are reported to have malignancy-like CEUS features both in human beings[23] and in dogs,[22] with a hyperenhancing wash-in (due to the intense arterial supply) and a quick wash-out (due to the lack of portal vessels).

The results reported here show that HCCs in cats have CEUS features similar to those reported in dogs.[5] In fact, most of the canine HCC cases showed inhomogeneous hyperenhancement (7/8) during wash-in, followed by inhomogeneous (6/8) hypoenhancement (7/8) during wash-out.[5] In addition, the CEUS characteristics (in particular homogeneity of the enhancement and wash-out time) of HCCs in human beings[24] are reported to be influenced by cellular differentiation. Such differences have not been evaluated here due to the relatively low number of HCCs included.

Lesion distribution within the present study population closely resembled the prevalence of hepatobiliary neoplasms already reported in cats, with BDA (45.4 per cent) being the most common, followed by BDC (36.3 per cent) and HCC (18.1 per cent).[1] The post hoc tests performed on TTE revealed that a high

Table 5 Diagnostic accuracy of TTE, TTP and wash-in rate. Ninety-five per cent confidence intervals are reported

Parameter	Cut-off value	AUC	Sensitivity	Specificity	PPV	NPV
TTE	≤4	0.781 (0.612 to 0.901)	45.75 (19.8 to 70.1)	95 (75.1 to 99.9)	87.5 (47.3 to 99.7)	67.9 (47.1 to 99.7)
TTP	≤15	0.758 (0.586 to 0.885)	75 (46.7 to 92.7)	75 (47.6 to 92.7)	70.6 (44 to 89.7)	78.9 (54.4 to 93.9)
Wash-in	≤4	0.781 (0.612 to 0.901)	93.8 (68.8 to 99.8)	50 (27.2 to 72.8)	60 (38.7 to 68.9)	90.9 (58.7 to 99.8)

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; TTE, time from injection to enhancement; TTP, time to peak.

statistical power was achieved, indicating that the same results might therefore be extended to the cat population.

The main limitation of this study is that cytopathology was used as a reference standard. The cytological differentiation between HCC and BDC is usually straightforward, but the differentiation between BDA and BDC only by means of cytology is reported to be challenging.[17] To overcome this limitation, the diagnosis of each included BDA was confirmed by at least two consecutive cytological examinations. Further studies, possibly using histopathology as reference standard, are advised in order to confirm the results presented here.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was conducted according to the Italian law D. Leg.vo 26/2014 (that transposes the EU directive 2010/63/EU). As the data used in this study were part of the routine clinical activity no ethical committee approval was needed. Informed consent regarding the treatment of personal data was obtained from the owners.

Data availability statement All data relevant to the study are included in the article.

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