Original Article

Journal of Feline Medicine and Surgery
2018, Vol. 20(1) 23–29

DOI: 10.1177/1098612X17697483
journals.sagepub.com/home/jfms

This paper was handled and processed by the European Editorial Office (ISFM) for publication in *JFMS*

Association between ultrasonographic appearance of splenic parenchyma and cytology in cats

Mileva Bertal[1], Eric Norman Carmel[2], Alessia Diana[3], Loic Desquilbet[4], Swan Specchi[5] and Pascaline Pey[1]

Abstract

Objectives The purpose of this study was to determine whether the presence of a splenic mass or a diffusely moth-eaten parenchyma on ultrasonographic scans could represent potential criteria of malignancy in the feline spleen.
Methods Feline patients with ultrasonographic images and cytological analysis of the spleen obtained by fine-needle aspiration were retrospectively included in a multicentre study.
Results One hundred and ninety-five cats met our inclusion criteria. There was a lack of agreement between the moth-eaten ultrasonographic appearance of the spleen and the presence of a malignant neoplasia on cytological analysis. The sensitivity and specificity of a moth-eaten parenchyma for predicting malignant neoplastic disease were 13.2% and 84.8%, respectively. The sensitivity and specificity of a splenic mass >1 cm for predicting malignant neoplastic disease were 21.0% and 94.7%, respectively. A marbled appearance of the splenic parenchyma was significantly more frequent among patients examined with a high-frequency transducer (11–18 MHz) than among those examined with a low-frequency transducer (6.6–10 MHz) (27.6% vs 11.1%, respectively; $P = 0.004$). Similarly, although not statistically significant, a moth-eaten parenchyma was more frequent in the high-frequency transducer group than in the low-frequency group (17.1% vs 8.9%, respectively; $P = 0.09$).
Conclusions and relevance Based on our findings, a moth-eaten ultrasonographic appearance of the spleen in cats does not necessarily reflect a lymphoma or another malignant neoplastic process on cytological analysis. The presence of a splenic mass >1 cm on ultrasound is suggestive of malignancy in cats. Finally, the transducer frequency must be taken into account when assessing the splenic parenchyma, as a high-frequency transducer seems to improve the detection of a marbled or moth-eaten parenchyma.

Accepted: 8 February 2017

Introduction

The ultrasonographic (US) appearance of splenic diseases in cats is understudied, and there are no US criteria of malignancy identified in the literature. Histological splenic lesions are uncommon in cats, with an estimated prevalence of 5%.[1] Among these, neoplastic conditions (primary and metastatic) reportedly account for 37% of the histological splenic abnormalities.[2]

Focal and diffuse US abnormalities of the feline spleen were previously described as non-specific.[3,4] In one report,[3] the most common description of splenic lymphoma was an enlarged, hypoechoic or nodular spleen with smooth margins; however, this was only found in 53% of the cats with lymphoma. The results from this previous study suggested that feline splenic disease was more likely to be neoplastic, compared with canine

[1]Department of Medical Imaging, Paris-Est University, National Veterinary School of Alfort, Maisons-Alfort, France
[2]Department of Medical Imaging, Veterinary Centre Laval, Laval, QC, Canada
[3]Department of Veterinary Medical Science, University of Bologna, Ozzano dell'Emilia, Italy
[4]Department of Biostatistics and Clinical Epidemiology, Paris-Est University, National Veterinary School of Alfort, Maisons-Alfort, France
[5]Department of Medical Imaging, Veterinary Institute of Novara, Granozzo con Monticello, Italy

Corresponding author:
Mileva Bertal DVM, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133 D4, 9820 Merelbeke, Belgium
Email: mileva_bertal@hotmail.com

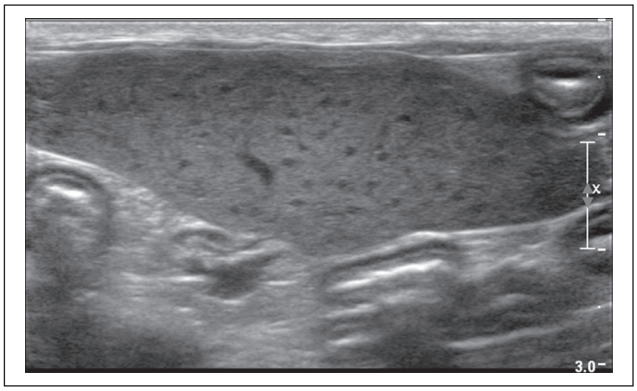

Figure 1 Appearance of a splenic moth-eaten parenchyma obtained with a 15 MHz linear transducer

splenic disease, with 73% of the US splenic changes being neoplastic in their population of cats.[3]

Cytological evaluation of the spleen (following percutaneous ultrasound-guided fine-needle aspiration) is a commonly used tool in the diagnostic work-up of veterinary patients.[5,6] Its indications include evaluation of nodules or focal masses, evaluation of diffuse splenomegaly or abnormal US echotexture, and staging or characterisation of systemic or multicentric haematopoietic disorders.[5–7]

The objective of this study was: (1) to determine the association between the frequency of the ultrasound transducer and the detection of a diffusely moth-eaten or marbled splenic parenchyma; (2) to evaluate the agreement between diffusely moth-eaten splenic echotexture and cytological diagnosis of splenic malignancy; and (3) to evaluate the sensitivity and specificity of a moth-eaten appearance of the splenic parenchyma and a splenic mass on US scans for predicting a neoplastic disease, based on cytological diagnosis.

Our hypotheses were that: (1) the use of a high-frequency transducer would improve the detection of a moth-eaten or marbled splenic parenchyma; (2) a diffuse moth-eaten appearance of the feline spleen in US images would not be suggestive of lymphoma or another malignant neoplasia; and (3) the presence of a splenic mass (>1 cm in diameter on US images) would be related to a malignant neoplastic condition on cytology.

Material and methods

Animals

The medical record databases of the Veterinary Teaching Hospital of the National Veterinary School of Alfort (ENVA) (France), of the Laval Veterinary Centre (CVL) (Canada) and of the Veterinary Institute of Novara (IVN) (Italy), were searched from March 2012 to September 2015 for cytological analyses of feline splenic aspirates. Inclusion criteria consisted of the following: (1) US examination of the abdomen, with good diagnostic quality US images of the spleen available for review in the database (PACS and Hospital Information System); (2) fine-needle aspiration (FNA) performed for the first time on the spleen of the patient; and (3) cytological analysis of the splenic aspirates performed within 2 weeks of US examination.

Data collected from the medical records included patient signalment, clinical signs, US findings, sedation protocol, transducer frequency and cytological diagnosis.

In the population from ENVA, the use of sedation and the choice of drugs were not systematically recorded, whereas in the populations from IVN and CVL, this was consistently detailed.

Ultrasonography

US examinations were performed with the cat in dorsal recumbency, with hair clipped, using a Philips IU 22 ultrasound machine (Philips) at ENVA; a Esaote MyLabTwice (Esaote) at CVL; and a Logiq9 (GE Medical Systems) at IVN. Various transducers were used (convex and linear), in B-mode, and their frequencies were recorded on the images. The US examinations were performed by a board-certified radiologist in CVL (ENC), by a board-certified radiologist (PP) or a university hospital radiologist in ENVA, and by a board-certified radiologist (SS) or resident in training supervised by a board-certified radiologist at IVN.

In the case of focal lesions, a minimum of two images were used, corresponding to the sagittal and transverse planes. In the case of a generalised parenchymal abnormality, the minimum number of images recorded was one single image, corresponding to the sagittal plane. It was considered as sufficient if the quality of this image was good.

The US images of the spleen were independently reviewed by three board-certified radiologists (PP, ENC, SS). The quality of the US images was evaluated regarding the spatial and contrast resolution, and the presence of artefacts, by the board-certified radiologist who reviewed and analysed the images. Patients were included if the quality of images was judged as sufficient.

Splenic parenchyma was classified according to the following criteria: normal echotexture, diffusely marbled, diffusely moth-eaten (Figure 1), diffusely hypoechoic, and presence of nodules (<1 cm in diameter) or mass(es) (>1 cm in diameter). In order to standardise the pattern characterisation, a consensus on the definition of the US images prior to their analysis was made between the three radiologists, to reach the lowest inter-observer variability.

A moth-eaten parenchyma was characterised by the presence of numerous small hypoechoic nodules throughout the spleen, causing a spotted echotexture. A marbled parenchyma was characterised by the presence of multiple overlapping ill-defined areas with heterogeneous echogenicity. This is distinct from the moth-eaten parenchyma where smaller separate individual lesions are seen.

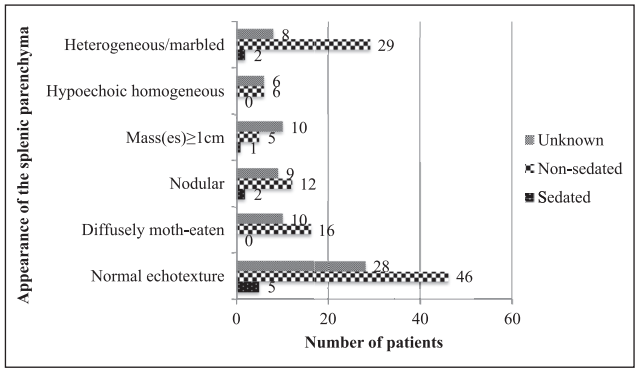

Figure 2 Ultrasonographic appearance of the splenic parenchyma and sedation status

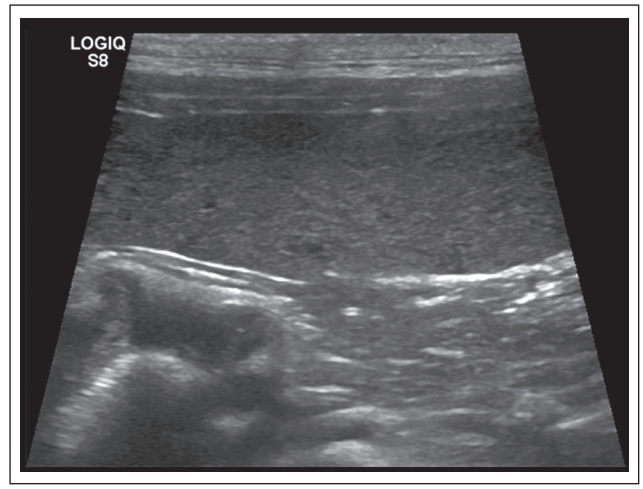

Figure 3 Appearance of a splenic marbled parenchyma obtained with a 15 MHz linear transducer

The original US reports were available and were used after the classification to confirm that no lesion had been missed on the recorded images of the patients.

Cytological analysis

FNA of the spleen was performed, in the absence of contraindication, when a focal lesion or diffuse parenchymal change was described, or, more seldomly, in the context of a staging, following a diagnosis of neoplasia. US guidance was consistently used, and in the case of focal lesions, these ones were specifically targeted. FNAs were achieved using 23 G, 25 mm needles and a non-aspiration technique.[8] Smears were coloured using a May–Grünwald and Giemsa stain before interpretation by a board-certified clinical pathologist. The number of slides per patients varied between two and four.

Statistical analysis

In order to take into account the sedation of the patient and the frequency of the transducer as potential bias in the US assessment of the splenic parenchyma, the effect of those parameters on the identification of a moth-eaten or marbled appearance of the spleen was studied in the first instance. Association between the transducer frequency (6.6–10 MHz vs 11–18 MHz) and the presence of either a moth-eaten or a marbled splenic parenchyma was tested using the χ^2 statistical test.

Agreement between moth-eaten US appearance and malignant neoplastic disease of the spleen determined by cytology was assessed via calculation of the kappa (κ) coefficient.[9] Briefly, a κ value of 1 indicated a perfect agreement between the two tests, and a value of 0 indicated agreement only by chance.[10] The κ statistic values indicated poor (<0.00), slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) or almost perfect (0.81–1.00) agreement.[10]

For this study, cytology was considered as the gold standard for identification of a neoplastic process in the spleen. Sensitivity, specificity, positive predictive value and negative predictive value of ultrasound for detection of neoplasia were determined.

Results

One hundred and ninety-five cats met the inclusion criteria. Breeds were domestic shorthair (n = 156), Oriental (n = 1), Bengal (n = 3), Siamese (n = 4), Turkish Angora (n = 1), Persian (n = 6), Norwegian Forest Cat (n = 1), British Shorthair (n = 1), Chartreux (n = 1), Maine Coon (n = 3), Exotic (n = 1), Abyssinian (n = 2), Ragdoll (n = 1), Siberian (n = 2), domestic longhair (n = 5), Burmese (n = 1), American (n = 1), Sphynx (n = 1) and unknown (n = 4). Their ages ranged from 1 year to 19 years (mean 9.3 years).

One hundred and thirty-one were male (98 neutered), 62 were female (51 neutered) and two were of unknown sex.

The US appearance of the splenic parenchyma is presented in Figure 2.

None of the sedated patients (10 cats) had a moth-eaten parenchyma (Figure 1) and two had a marbled splenic parenchyma (Figure 3).

A marbled appearance of the splenic parenchyma was significantly more frequent among patients examined with a high-frequency transducer (11–18 MHz) than among those examined with a low-frequency transducer (6.6–10 MHz) (27.6% vs 11.1; $P = 0.004$) (Table 1). Similarly, although not statistically significant, a moth-eaten parenchyma (Figure 1) was more frequent in the high-frequency transducer group than in the low-frequency group (17.1% vs 8.9%; $P = 0.09$). Only seven cats had images recorded with both a high- and a low-frequency transducer (8 MHz and 12 MHz). In these cases, there was an agreement in the appearance of the spleen between

Table 1 Comparison of the splenic parenchyma between the group of cats examined with a low-frequency transducer (6.6–10 MHz) and with a high-frequency transducer (11–18 MHz)

	Moth-eaten parenchyma		Marbled parenchyma		Total
	Yes	No	Yes	No	
Low frequencies (6.6–10 MHz)	8 (8.9%)	82	10 (11.1%)	80	90
High frequencies (11–18 MHz)	18 (17.1%)	87	29 (27.6%)	76	105
P value	0.09		0.004		
Total	26 (13.3%)	169	39 (20.0%)	156	195

Table 2 Agreement between a moth-eaten appearance of the spleen and a malignant neoplastic disease

		Malignant neoplastic disease		
		Present	Absent	Total
Moth-eaten appearance	Present	5	20	25
	Absent	33	112	145
	Total	38	132	170
Kappa (95% CI)			–0.02 (–0.17 to 0.12)	
P value			0.76	
Interpretation of kappa value				
<0			Poor	

CI = confidence interval

both transducers. Most notably, one moth-eaten parenchyma and one marbled parenchyma were visualised with both transducers.

Ultrasound-guided FNA of the spleen was diagnostic in 170/195 cases sampled (87.1%).

Neoplastic infiltration of the spleen was detected in 38/170 (22.3%) cats. A moth-eaten appearance of the spleen was found in 25/170 (14.7%) cats (Table 2). Of these 25 cats, five had a cytological diagnosis of malignant neoplastic disease (three lymphoma, one metastatic carcinoma, one histiocytosis) and 20 had a diagnosis of benign or non-neoplastic disease (Figure 4). The κ coefficient revealed a lack of agreement between the moth-eaten US appearance of the splenic parenchyma and the presence of a splenic malignant neoplasia ($\kappa = -0.02$). The specificity, sensitivity, positive predictive value and negative predictive value of a moth-eaten parenchyma for predicting malignant neoplastic disease were 84.8%, 13.2%, 20.0% and 77.2%, respectively.

To investigate our third hypothesis, US findings were compared with cytological diagnosis to determine the sensitivity and specificity of US images as a predictor of malignant neoplasia in cats when a mass >1 cm in diameter (Figure 5) is present in the splenic parenchyma. A splenic mass was found in 15/170 (8.8%) cats (Table 3). Among these, eight had a diagnosis of malignant neoplasia (four carcinomas, two lymphomas, one haemangiosarcoma, one multiple myeloma) and seven had a diagnosis of non-neoplastic disease (Figure 4). The specificity, sensitivity, positive predictive value and negative predictive value of a splenic mass for predicting malignant neoplastic disease were 94.7%, 21.0%, 53.3% and 80.6%, respectively.

Discussion

Our results showed a lack of agreement between the US moth-eaten appearance of the spleen and the presence of a malignant neoplastic disease on splenic cytology in cats. Therefore, as we hypothesised, a moth-eaten appearance of the spleen identified on US examination in cats was not predictive of lymphoma (or any other malignant neoplasia), in contrast to what has been described in dogs.[11]

The US appearance of the spleen in dogs with splenic lymphoma includes a moth-eaten, coarse parenchyma, ill-defined hypoechoic areas, hypoechoic nodules and the presence of a complex mass.[4,11] In a study about characterisation of the hepatic and splenic changes in dogs with lymphoma, a lymphoma was identified on cytology in all evaluated cats with a moth-eaten spleen.[11] When the spleen was described as moth-eaten, the positive predictive value of ultrasound for the detection of lymphoma was 100%.[11] Therefore, no analogy can be made between the conclusions of a moth-eaten appearance of the spleen in dogs and in cats. In cats, an important variability in the US appearance of lymphoma was previously described.[3] In this

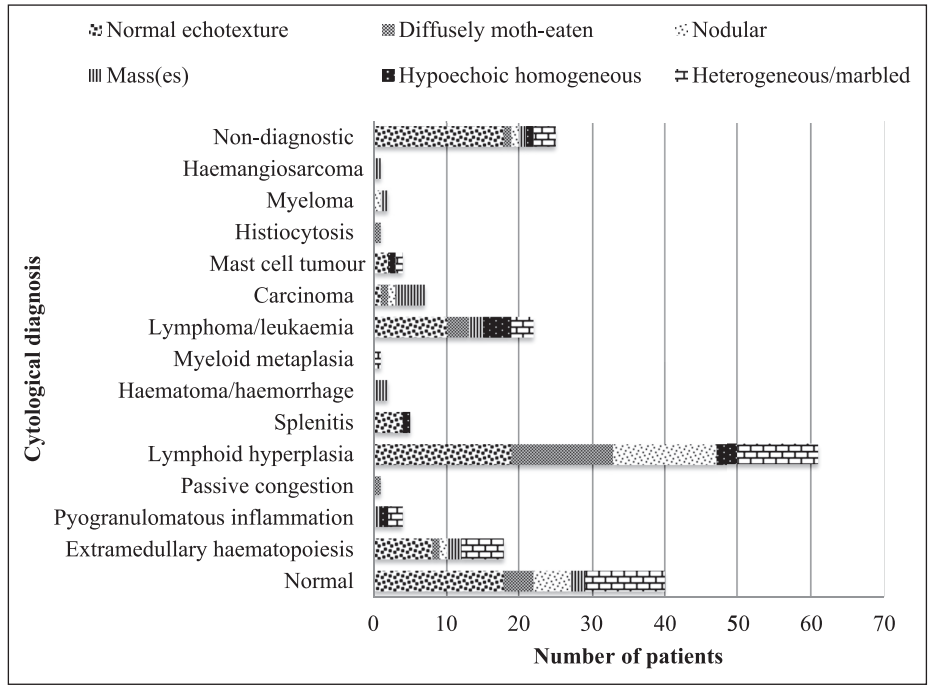

Figure 4 Prevalence of each ultrasonographic appearance per cytological diagnosis in the population of the present study

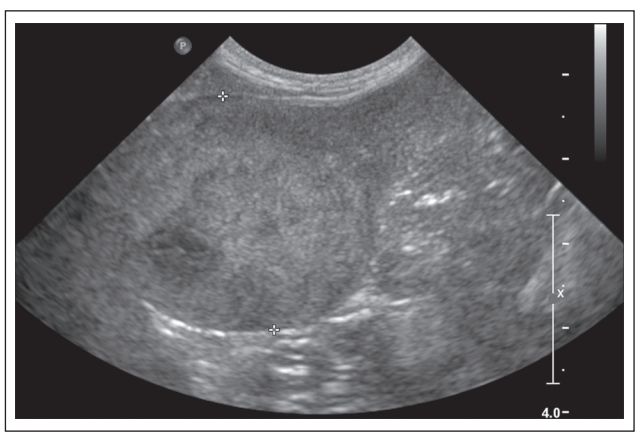

Figure 5 Appearance of a splenic mass (delimited by calipers) with an 8 MHz curvilnear transducer

Table 3 Ultrasonographic vs cytological findings in the case of a splenic mass

		Malignant neoplastic disease		
		Present	Absent	Total
Splenic mass >1 cm	Present	8	7	15
	Absent	30	125	155
	Total	38	132	170

study, a mottled splenic parenchyma was observed in cats with a cytological diagnosis of mast cell tumour, lymphoproliferative and myeloproliferative diseases, extramedullary haematopoiesis and lymphoid hyperplasia.[3] In our population, a moth-eaten appearance of the spleen was observed in cats diagnosed with lymphoid hyperplasia, extramedullary haematopoiesis, passive congestion, lymphoma, carcinoma and histiocytosis, as well as in normal cats.

The proportion of cats with a moth-eaten or a marbled appearance of the splenic parenchyma was not higher in those that received sedation prior to the US examination than in non-sedated cats. This suggests that the sedation does not have an effect on the moth-eaten or the marbled appearance of the splenic parenchyma, but the low number of sedated cats makes it difficult to draw strong conclusions. Also, a comparison of the appearance of the spleen before and after sedation would be necessary to determine whether sedation modifies the US assessment of splenic parenchyma.

A significantly higher proportion of cats with a marbled appearance of the spleen was observed with a high-frequency transducer than with a low-frequency transducer ($P = 0.004$). The same tendency was noted regarding the percentage of cats presenting a moth-eaten parenchyma of the spleen, but the difference was not statistically significant ($P = 0.09$). Because of the low number of moth-eaten splenic parenchyma (n = 26) compared with the marbled parenchyma (n = 39), this lack of statistical significance may be due to a weak statistical power. This result suggests that using a high-frequency transducer may improve the detection of the marbled and moth-eaten splenic parenchyma. Nonetheless, it must be interpreted with caution, as the proportion of

cats with these US findings may have been different in the groups of cats examined with a high-frequency and with a low-frequency transducer. Nevertheless, the similar tendency noted for these two patterns leads us to suspect that this finding was not only a consequence of unbalanced groups, but also that the transducer frequency may affect the assessment of the splenic parenchyma. Moreover, this is consistent with the fact that higher US frequencies improve the axial resolution of the images, and hence we believe that a moth-eaten pattern may be more frequently and easily seen with higher-frequency transducers.

The calculated specificity of a splenic mass seen on ultrasound for predicting a malignant neoplastic disease was 94.7%. This specificity value indicates that ultrasound is a useful test for the detection of splenic malignant neoplasia when a splenic mass is identified in cats. This result contrasts with a previous study,[3] where a higher percentage of cats with non-neoplastic splenic disease had splenic masses compared with the group with splenic neoplasia. This can be due, in part, to the dimensions of the masses included in our study. We chose to select the cats with masses >1 cm in diameter, in order to differentiate with splenic nodules, which are known to be non-specific.[3] Considering that the spleen should not exceed 1 cm in thickness in cats,[12,13] and that by definition a mass deforms the contours of an organ, we decided to use the threshold of 1 cm to distinguish masses from nodules. The cytological diagnoses of the cats with a splenic mass included carcinoma, lymphoma, multiple myeloma, haemangiosarcoma, pyogranulomatous inflammation, haematoma, extramedullary haematopoiesis and normal spleen.

In our study, cytological examination was used as the gold standard for the diagnosis of splenic neoplasia. Ideally, the gold standard for evaluating splenic lesions should have been diagnostic splenectomy, because it allows collection of large tissue samples for histopathological assessment. To our knowledge, the sensitivity and specificity of cytology for the diagnosis of splenic neoplasia is unknown in cats, owing to the limited access to full-organ biopsy in this species for definitive diagnosis.

Several authors have studied the correlation between cytology and histology (after surgical biopsy or necropsy) in dogs and cats, but the populations of cats were usually very small. The results are widely variable, with complete cytopathological and histopathological agreement ranging from 100% to 59%.[14,15] The latter had an overall agreement (complete and partial) of 88%.[15]

Another study focused on the correlation between ultrasound-guided FNA and needle-core biopsy of the canine spleen.[16] FNA was shown to be as beneficial as needle-core biopsy alone for diagnosing splenic neoplasia, as fewer false-negative results were obtained cytologically than histologically. FNA has inherent advantages over needle-core biopsy, including the ability to sample deeper lesions, sample perivascular lesions safely and allow multiple different passes through a single lesion.[16] However, histological examination provides additional information about tissue architecture, allows assessment of certain nuclear features important in the classification, and offers the possibility to use immunocytochemical techniques. Their use in combination can provide useful complementary information that improves neoplastic detection and tumour subclassification.[16]

The predictive value of splenic cytology depends, among others, on the prevalence of different types of splenic disease.[7,17] Haematopoietic hyperplasia and neoplasia are likely to be successfully identified by splenic cytology because of the ease with which haemic cells exfoliate and the superiority of the routinely used stains for identifying, characterising and differentiating cells of haemic origin, including round cells.[7] Mesenchymal proliferation and sarcomas are less likely to exfoliate, although haemorrhage and necrosis associated with sarcomas may be detected.[7] In a survey of 455 cats with splenic disease, 67% had haemic disorders, including haematopoietic neoplasia, reactive hyperplasia, extramedullary haematopoiesis, congestion and inflammation.[2] Considering the high prevalence of haemolymphatic disease in the spleen in cats (vs dogs), splenic cytology would be expected to have high predictive value in cats.[7]

However, in comparison with dogs, the diagnosis of lymphoma is more challenging in cats because it more commonly affects extranodal sites.[18] Diffuse lymphomas composed of small or intermediate cells can be difficult to diagnose by cytology, because the cells more closely resemble benign lymphocytes.[18] Specifically, there is overlap in the cytological appearance between benign splenic reactive lymphoid hyperplasia and malignant small cell lymphoma or low-grade lymphoma. In those situations, histopathology is essential in differentiating some benign from malignant processes that appear similar cytologically.[16] They represent approximately 10% of lymphomas of cats, which are composed of small, relatively well-differentiated, neoplastic lymphoid cells and can be histologically described as low grade.[19]

For these reasons, and as the use of histology of the spleen is exceptional in our practices, we considered cytology as a relatively reliable method for the diagnosis of splenic disease in cats. Unfortunately, there were no final diagnoses, based on splenectomy or necropsy, which is a limitation of our study. Furthermore, although the cytological diagnoses were made by a board-certified clinical pathologist, they should ideally have been reviewed independently by another pathologist, which was not the case in our study.

Another limitation is the retrospective nature of the study. The interpretation of the US images was mainly undertaken using still images, which is not optimal for the interpretation of the echotexture, and for the identification of a moth-eaten appearance. Likewise, the size of the spleen could not be integrated as an additional

criterion in the detection of a neoplastic disease, because it was retrospectively impossible to get a standardised measurement of the spleen. A previous study on healthy cats showed that the measurements of the spleen can vary based on the area interrogated, and that the protocol recommended for consistent evaluation of the spleen in the cat requires three specific measurements (the head and tail obtained from a transverse plane and the body from a sagittal plane).[12] The assessment of the size of the spleen in our study would have been too subjective and not reliably comparable.

Moreover, a different ultrasound machine was used in each of the three veterinary centres, and all the images were reviewed by the three board-certified radiologists independently, leading to a subjective evaluation of the images by three different observers. However, a prior consensus on pattern characterisation was reached between the three board-certified radiologists, in order to minimise the inter-observer variability of interpretation.

Finally, additional US criteria such as the size of the spleen and the appearance of other structures such as the liver or the regional lymph nodes were not taken into consideration in the present study. Characterisation of splenic lesions must always be undertaken in the light of the entire US examination and further studies integrating splenic changes with additional US criteria may be useful in the future.

Conclusions

Based on our findings, a moth-eaten US appearance of the spleen in cats does not necessarily reflect a lymphoma or another malignant neoplastic process on cytological analysis. The presence of a splenic mass >1 cm on US images is suggestive of malignancy in cats based on cytological examination. Finally, the transducer frequency must be taken into account when assessing the splenic parenchyma, as a high-frequency transducer seems to improve the detection of a marbled or moth-eaten parenchyma.

Acknowledgements We thank the Department of Anatomy and Pathology of the ENVA, especially Edouard Reyes Gomez for his help and advice. We also thank Simona Morabito from the IVN for her help in collecting cat data.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding The authors received no financial support for the research, authorship, and/or publication of this article.

References

1 Ismahel J and Howell JM. **Observations on the pathology of the spleen of the cat**. *J Small Anim Pract* 1968; 9: 7–13.
2 Spangler WL and Culbertson MR. **Prevalence and type of splenic diseases in cats: 455 cases (1985–1991)**. *J Am Vet Med Assoc* 1992; 201: 773–776.
3 Hanson JA, Papageorges M, Girard E, et al. **Ultrasonographic appearance of splenic disease in 101 cats**. *Vet Radiol Ultrasound* 2001; 42: 441–445.
4 Lamb CR, Hartzband LE, Tidwell AS, et al. **Ultrasonographic findings in hepatic and splenic lymphosarcoma in dogs and cats**. *Vet Radiol Ultrasound* 1991; 32: 117–120.
5 Ballegeer EA, Forrest LJ, Dickinson RM, et al. **Correlation of ultrasonographic appearance of lesions and cytologic and histologic diagnoses in splenic aspirates from dogs and cats: 32 cases (2002–2005)**. *J Am Vet Med Assoc* 2007; 230: 690–696.
6 Nerschbach V, Eberle N, Joetzke AE, et al. **Splenic and hepatic ultrasound and cytology in canine lymphoma: effects of findings on stage migration and assessment of prognosis**. *Vet Comparative Oncol* 2016; 14 Suppl 1: 82–94.
7 Christopher MM. **Cytology of the spleen**. *Vet Clin North Am Small Anim Pract* 2003; 33: 135–152.
8 LeBlanc CJ, Head LL and Fry MM. **Comparison of aspiration and nonaspiration techniques for obtaining cytologic samples from the canine and feline spleen**. *Vet Clin Pathol* 2009; 38: 242–246.
9 Cohen JA. **Coefficient of agreement for nominal scales**. *Educ Psychol Meas* 1960; 20: 37–46.
10 Landis JR and Koch GG. **The measurement of observer agreement for categorical data**. *Biometrics* 1977; 33: 159–174.
11 Crabtree AC, Spangler E, Beard D, et al. **Diagnostic accuracy of gray-scale ultrasonography for the detection of hepatic and splenic lymphoma in dogs**. *Vet Radiol Ultrasound* 2010; 51: 661–664.
12 Sayre RS and Spaulding KA. **Formulation of a standardized protocol and determination of the size and appearance of the spleen in healthy cats**. *J Feline Med Surg* 2014; 16: 326–332.
13 Reese SL, Zekas LJ, Iazbik MC, et al. **Effect of sevoflurane anesthesia and blood donation on the sonographic appearance of the spleen in 60 healthy cats**. *Vet Radiol Ultrasound* 2013; 54: 168–175.
14 O'Keefe DA and Couto CG. **Fine-needle aspiration of the spleen as an aid in the diagnosis of splenomegaly**. *J Vet Intern Med* 1987; 1: 102–109.
15 Christensen NI, Canfield PJ, Martin PA, et al. **Cytopathological and histopathological diagnosis of canine splenic disorders**. *Aust Vet J* 2009; 87: 175–181.
16 Watson AT, Penninck D, Knoll JS, et al. **Safety and correlation of test results of combined ultrasound-guided fine-needle aspiration and needle core biopsy of the canine spleen**. *Vet Radiol Ultrasound* 2011; 52: 317–322.
17 Civardi G, Vallisa D, Bertè R, et al. **Ultrasound-guided fine needle biopsy of the spleen: high clinical efficacy and low risk in a multicenter Italian study**. *Am J Hematol* 2001; 67: 93–99.
18 Burkhard MJ and Bienzle D. **Making sense of lymphoma diagnostics in small animal patients**. *Vet Clin North Am Small Anim Pract* 2013; 43: 1331–1347.
19 Valli VE, Jacobs RM, Norris A, et al. **The histologic classification of 602 cases of feline lymphoproliferative disease using the National Cancer Institute working formulation**. *J Vet Diagn Invest* 2000; 12: 295–306.