

Acoustic radiation force impulse (ARFI) elastography of the spleen in healthy adult cats – a preliminary study

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OBJECTIVES: To evaluate the splenic stiffness of healthy adult cats using acoustic radiation force impulse elastography to determine the quality (greyscale images and tissue deformity) and quantity (shear velocity) standards.

METHODS: Fifteen healthy, adult shorthair cats were selected. The echotexture, echogenicity, size and edges of the spleen were assessed via mode-B ultrasound. Using qualitative elastography, specific portions of the spleen were evaluated according to homogeneity, presence of deformities and white and dark regions. The shear velocities in different portions of the spleen were quantitatively evaluated.

RESULTS: The echotexture, echogenicity, size and edges of the spleen were normal on B-mode ultrasound in all cats. On qualitative elastography, the evaluated splenic portions were not deformable, and the images presented as homogeneous dark areas. On quantitative elastography, the mean shear velocity values were 1.98 m/s for the head portion, 1.77 m/s for the body portion and 2.03 m/s for the tail portion. These were not significantly different.

CLINICAL SIGNIFICANCE: Quantitative and qualitative acoustic radiation force impulse elastography of the spleen in healthy adult cats was easily implemented and this study may provide baseline data for this organ to allow the future use of this technique in evaluating cats with splenic disease.

Journal of Small Animal Practice (2015) **56**, 180–183
DOI: 10.1111/jsap.12307

Accepted: 17 October 2014; Published online: 5 December 2014

INTRODUCTION

Elastography is a promising ultrasound technique that evaluates tissue elasticity. Various methods for assessing tissue elasticity have been proposed, such as compression, acoustic radiation force impulse (ARFI) and real-time shear velocity (RSV) (White *et al.* 2014).

The elastography by compression method is based on the estimated time delay between gated precompression and postcompression echo signals (Destounis & Gruttadauria 2013). In this technique, the tissue being evaluated is subjected to a force,

and the imaging system then measures the response of the tissue to this force, either by mechanical or automatic compression (Săftoiu *et al.* 2007, Hoskins 2012). Regarding its advantages, this technique is commercially accessible and has been validated as a useful medical tool. Regarding its disadvantages, the technique is ultrasonographer dependent and is sensitive to body conformation (weight and abdominal depth), particularly when evaluating deeper organs or obese patients (Carvalho & Chammas 2013).

ARFI elastography is safe and non-invasive and provides both quantitative and qualitative measures of tissue rigidity (Dudea *et al.* 2011). This technique is based on the propagation of a force

capable of causing the displacement of the tissue without the need for manual compression. In this method, the measurements cease to be subjective because they are not the result of variation of the compression force applied by the operator. Therefore, compared to other methods, these measurements are more accurate with less inter-observer variation and with greater reproducibility. One disadvantage is the high cost for the acquisition of the equipment (Carvalho & Chammas 2013).

For qualitative ARFI, short acoustic pulses and high intensities are utilised to deform the elements of the tissue and create a static map (elastogram) of the relative stiffness of the tissue. This method provides a greyscale map that demonstrates the relative stiffness of the tissues of the studied region, as compared to the corresponding conventional ultrasound image. In general, the lighter areas represent the more deformable tissues (Goddi *et al.* 2012).

The quantitative study uses a primary acoustic impulse towards a region of interest, promoting the formation of pressure waves capable of deforming the tissues and therefore raising the speed of propagation of the pressure waves (shear). The velocity of propagation and the attenuation of waves are related to the rigidity and viscoelasticity of the tissue. The waves have a higher velocity in rigid tissues (Comstock 2011).

In human medicine, ARFI elastography has been used principally for the identification and differentiation of mammary tumours, diagnosis of prostate tumours and focal fibrotic lesions (Srinivasan *et al.* 2004), evaluation of the myocardium (Nightingale 2011) and evaluation of the musculoskeletal and gastrointestinal systems (Palmeri & Nightingale 2011). In veterinary medicine, the ARFI technique is novel and experimental and has been used to evaluate focal liver lesions in rats (Carvalho *et al.* 2012), mammary tumours in female dogs (Feliciano *et al.* 2014) and the spleen, liver and kidneys of adult dogs (Holdsworth *et al.* 2014).

There are several previous studies on splenic ultrasound changes in cats. According to Hanson *et al.* (2001), ultrasonographic findings of splenic alterations in cats are not always specific for differentiation of malignant and benign disease, and the definitive diagnosis requires cytological or histological evaluation, both of which are considered to be more invasive methods.

In feline medicine, a pilot study (White *et al.* 2014) used the combination of B-mode ultrasound and elastography by compression to aid in the diagnosis of alterations in abdominal organs, including the spleen. Considering this pilot information and the reports of ARFI for splenic tissue evaluation in small animals, the aims of the present study were to describe the technique of ARFI elastography in the evaluation of the spleen of adult cats, to evaluate the stiffness of healthy splenic parenchyma, and to determine the quality and quantity (shear velocity) standards of the ARFI technique, which to the authors' knowledge have not yet been described in veterinary medicine.

MATERIALS AND METHODS

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the Faculdade de Ciências

Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Campus Jaboticabal, Brazil (protocol no 018.897/13). Fifteen healthy, adult, entire male, domestic, shorthair cats, three to five years of age (mean age = 4 ± 0.78 years) and weighing between 2.7 and 4.9 kg (mean = 3.82 ± 0.7 kg) were selected for this study following a normal physical, haematological examination, biochemical profile, urinalysis and urinary protein to creatinine ratio (UPC) evaluation.

After the animals were selected, the abdominal fur was shaved to allow for the ultrasonography. Before the examination, coupling gel was applied to the skin. No sedation was required.

The ultrasonography was performed by a single, experienced ultrasonographer. B-Mode ultrasonography was performed with a 9.0 MHz linear matrix transducer by using ACUSON S2000/SIEMENS ultrasound equipment (Siemens, Munich, Germany). The echotexture (homogeneous or heterogeneous), echogenicity (hypoechoic, hyperechoic or mixed) of the parenchyma, size (increased, decreased or normal) and edge (rounded or fine) of the spleen were assessed and categorised (subjective evaluation).

For the elastography, software for qualitative and quantitative analysis was compared using the ARFI method (Virtual Touch Tissue Quantification) and a 9.0 MHz linear matrix transducer (Syversveen *et al.* 2011, Feliciano *et al.* 2014).

After performing B-mode ultrasonography, the qualitative ARFI technique was applied, resulting in the formation of greyscale images of the cranial (head), medial (body) and caudal (tail) portions of the spleen. The images were evaluated with specific focus on the presence of deformities, white areas (indicative of more elastic tissue that is less rigid, softer and more deformable) and dark areas (more rigid, harder and not deformable regions within the spleen).

Quantitative evaluation was also performed after scanning the spleen with the B-mode ultrasound. The function for obtaining the shear velocity was activated, and the calliper was positioned in the spleen parenchyma; the fixed region of interest box dimensions were 10 mm × 10 mm. Six measurements in each position (head, body and tail) of the spleen were obtained with depth between 0.8 and 1.1 cm and used to determine the shear velocities [mean and standard deviation (sd)] (Fig 1).

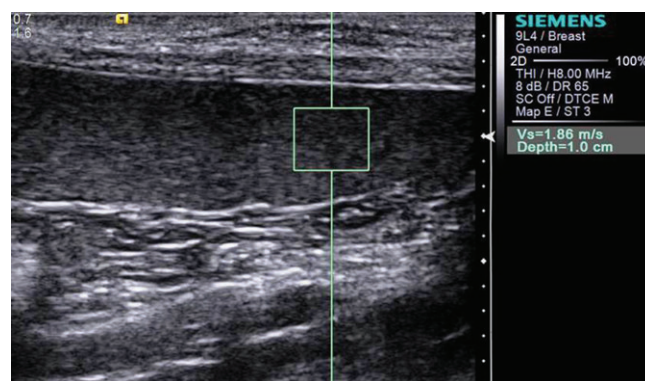


FIG 1. Ultrasound image of a feline spleen during its quantitative ARFI evaluation of the caudal portion (tail). Note the measurement of shear velocity of splenic tissue, with the presence of calliper the portion assessed

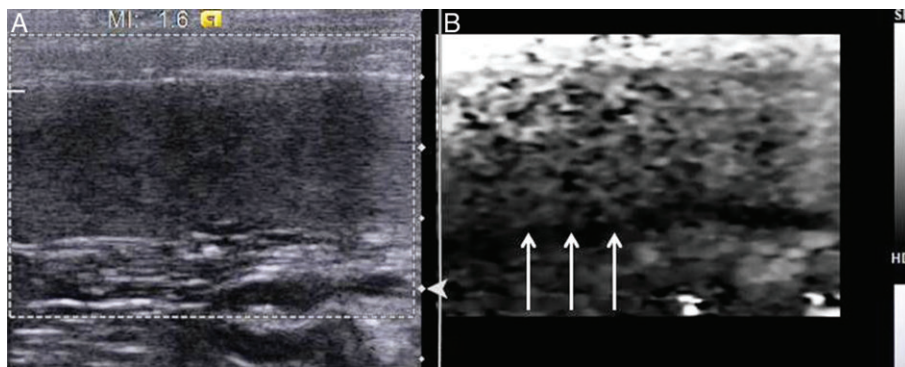


FIG 2. Ultrasound image of feline spleen during its qualitative ARFI evaluation. In (A) the B-mode image of the medial portion and in (B) the image of elastography of the spleen (arrows) with the characterization of the stiffness of splenic tissue in greyscale and evaluation of the deformity

The data were analysed for normality of residuals and homogeneity of variances (F test). PROC MEANS-SASTM and Graph-Pad Prisma 4TM were used for the analysis. The significance level was set $P < 0.05$ for all tests. Raw and transformed means were evaluated by an analysis of variance. In addition, a t-test for paired samples was applied for all shear velocities and depth from the different splenic portions. In addition, using the Pearson test, a correlation between the weight of the animals and the shear rates obtained in the various portions of the feline spleen was evaluated. Qualitative data (echotexture, echogenicity, size and edges; greyscale images and presence of deformities) were described only.

RESULTS

Assessment of the spleen in all cats using B-mode ultrasound showed no abnormalities in shape, size, borders or parenchyma.

Qualitative and quantitative elastography of the cranial, middle and caudal portions of the spleen in felines were performed without difficulty. On qualitative elastography, the evaluated splenic portions were not deformable, and the images presented as homogenous dark areas (Fig 2).

Using quantitative elastography, the mean shear velocity values were 1.98 m/s (95% confidence interval: 1.70 and 2.26 m/s) for the cranial portion, 1.77 m/s (95% confidence interval: 1.54 and 1.99 m/s) for the medial portion, and 2.03 m/s (95% confidence interval: 1.79 and 2.26 m/s) for the caudal portion of the spleen. None of these values were significantly ($P = 0.2435$) different (Fig 3). Additionally, it was found that the depth used for acquiring the velocities in the different portions of the feline spleen did not represent a significant difference between the portions analysed ($P = 0.5818$).

Some values obtained for shear velocity in different portions of the feline spleen could be considered “outliers” because some samples exceeded the obtained confidence interval (Fig 3). However, these values were maintained in order to obtain a maximum value for shear velocity that was more accurate and real.

No significant correlation was found between the body-weight and shear velocity values in splenic tissue ($P = 0.50707$ and $R^2 = 0.0275$ for the cranial portion; $P = 0.8178$ and $R^2 = 0.0046$ for

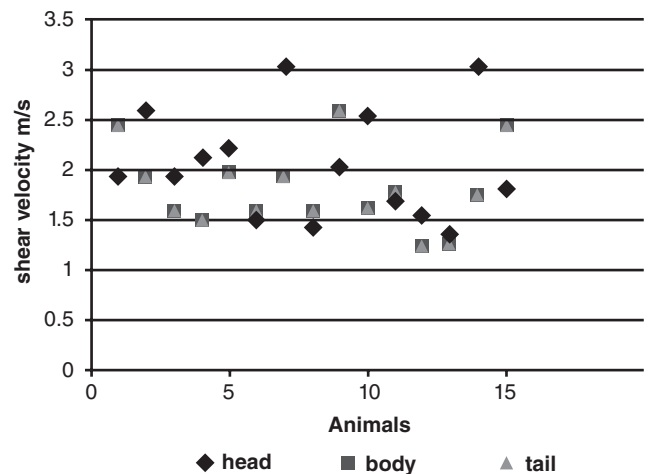


FIG 3. Graphic illustration showing the distribution of values for shear rate obtained in splenic portions of adult cats and the presence of outliers present in this evaluation

the medial portion and $P = 0.1183$ and $R^2 = 0.1909$ for the caudal portion).

DISCUSSION

The spleen may be affected by inflammatory, circulatory, haematological and neoplastic diseases, either as a primary site or as a site of metastasis (Harari 1999). The diagnosis of these alterations is of paramount importance for small animals because the spleen is involved in lymphatic, immune, circulatory and haematopoietic function (Morris & Dobson 2007). This study presents a new technique for the study of splenic parenchyma in small animals.

B-Mode ultrasound is useful for the detection of splenomegaly or splenic masses in dogs and cats, and assists in biopsies or fine needle aspirates (Couto 1992). However, splenic ultrasonography is not a specific diagnostic technique because of the difficulty of identifying diffuse abnormalities and differentiating between malignant and benign tissues (Vescoci *et al.* 2009, Garcia *et al.* 2012). Considering the preliminary results obtained in the evaluation of mammary neoplasms in bitches by the ARFI

method (rigid and non-deformable mammary neoplastic tissue and high shear velocity are indicative of malignity) (Feliciano *et al.* 2014), the novel data obtained in this study may aid veterinary clinicians when diagnosing splenic abnormalities in cats in the future.

In the present study, the splenic parenchyma was rigid and homogeneous and was not deformable in relation to the adjacent tissue for qualitative elastography. Therefore, this technique could be performed to detect the intraparenchymal areas (hard/soft or deformable/not deformable) that might alter the overall homogeneity.

To minimise the effects of body conformation (weight and abdominal depth), age and sex cited by Holdsworth *et al.* (2014) in elastography of canine spleens, male animals of the same breed, with little variation in weight and age, were used in the present study.

The quantitative ARFI verified that the shear velocity values in the splenic portions were similar. The results obtained for shear velocity in adult feline spleens (all and portions) were similar to those observed in healthy human subjects – mean values were 2.04 ± 0.28 m/s (Bota *et al.* 2010). These results were also similar to the values found in adult dogs for the interquartile range: 1.59 to 2.4 m/s at 0 to 2 cm (depth) and 1.45 to 1.94 m/s at 2 to 4 cm (depth) (Holdsworth *et al.* 2014).

These quantitative values obtained by ARFI of the spleen in adult cats are, to the authors' knowledge, the first that have been described in feline medicine. Studies in animals with splenic alterations should be performed to further evaluate the applicability of ARFI as a diagnostic technique. The technique has proven its applicability in mammary tissue in humans (Bai *et al.* 2012, Tozaki *et al.* 2011) and bitches (Feliciano *et al.* 2014), in which high shear rates (compared to healthy state mammary tissue) are related to greater mammary tissue stiffness and malignancy.

There are some important limitations in this study. Poor patient compliance may hamper the acquisition of measurements. Although sedation was not used in this study, the subjects were kept in a quiet environment before examination, and minimal restraint was used. A small number of animals were studied, with little breed, age or sex variation. However, this pilot study provides important information about the validation of the technique and about obtaining reference values for adult male cats. However, as noted by Holdsworth *et al.* (2014) in their study with dogs, it is necessary to conduct a study with a larger number of animals and, specifically, to evaluate cats of different breeds, weights, sex and age ranges to verify the interference of these variables on the findings of elastography in the feline spleen.

It is believed that after the standardisation of the reference values for splenic elastography in healthy cats and with a larger sample size, the differences in values of shear velocity from damaged tissues can be evaluated for the applicability of ARFI elastography in differentiating benign and malignant splenic abnormalities in cats.

Quantitative and qualitative ARFI elastography of the spleen in healthy adult cats was easily implemented and may provide

baseline data for this organ to allow the use of ARFI as a diagnostic tool for evaluating splenic abnormalities in sick cats.

Acknowledgements

The authors thank FAPESP for a research grant and research for young investigators (processes 2012/16635-2 and 2013/06443-1).

Conflict of interest

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

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