

Acoustic radiation force impulse elastography of the spleen in healthy dogs of different ages

M. C. MARONEZI*, M. A. R. FELICIANO†, L. Z. CRIVELLENTI*, A. P. R. SIMÕES†, P. M. BARTLEWSKI‡, I. GILL‡, J. C. C. CANOLA* AND W. R. R. VICENTE†

*Department of Surgery Veterinary, College of Agricultural and Veterinary Sciences, Sao Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil

†Department of Animal Reproduction, College of Agricultural and Veterinary Sciences, Sao Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil

‡Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

OBJECTIVES: To determine the elastographic characteristics of splenic parenchyma in clinically healthy dogs of various ages in order to establish preliminary qualitative and quantitative standards/reference intervals for this technique.

MATERIALS AND METHODS: Thirty three healthy dogs categorized as young, adult and elderly were used. Splenic echotexture, echogenicity, size and ages were assessed with B-mode ultrasonography. Using qualitative elastography, the spleen (head, body and tail) was examined for homogeneity and presence of deformities. Shear velocities in different splenic segments were then quantitatively evaluated.

RESULTS: All splenic segments visualised with the B-mode ultrasonography appeared normal. Different splenic segments examined with qualitative elastography were free of any detectable malformations and the images appeared as homogeneous dark areas. The mean shear velocity values were 2.32 m/s for head, 2.16 m/s for body and 2.25 m/s for tail of the spleen ($P=0.40$), and they did not vary between the different age groups ($P>0.05$).

CLINICAL SIGNIFICANCE: Quantitative and qualitative ARFI elastography of the spleen in clinically healthy dogs differing in age could be easily performed, and it may aid in the diagnosis and evaluation of splenic abnormalities routinely assessed in veterinary practice with B-mode ultrasonography.

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INTRODUCTION

Currently, there is an increasing interest in the non-invasive assessment of the viscoelastic properties of internal organs and tissues using novel ultrasonography-based imaging modalities. Ultrasonographic tissue-strain analyses can be performed with (elasticity imaging) or without compression (acoustic radiation force impulse (ARFI) imaging; Gallotti *et al.* 2010). The ARFI technique is safe and non-invasive, and it provides quantitative and qualitative measures of tissue rigidity with reduced inter-observer variability compared with other diagnostic techniques (Dudea *et al.* 2011, Feliciano *et al.* 2014).

In qualitative ARFI, short acoustic pulses of high intensities are utilised to transiently deform the fragments of the tissue and create a greyscale static map or matrix (elastogram) representing the relative stiffness of the area of interest. In general, the lighter areas represent the more deformable tissues (Goddi *et al.* 2012). Alternatively, the quantitative approach to ARFI utilises a primary acoustic impulse sent towards a region of interest and promoting the formation of pressure waves capable of deforming the tissues to raise the speed of the wave propagation (shear velocity). The wave velocity and the attenuation of acoustic pressure waves are both related to the rigidity and viscoelasticity of the tissue; the waves have a greater velocity in rigid tissues (Comstock 2011).

In humans, ARFI elastography has primarily been used to identify and differentiate various types of mammary and prostate gland tumours. In addition, splenic elastography has been employed in the diagnosis of concurrent hepatic disorders (Bota *et al.* 2010, Dudea *et al.* 2011, Gao *et al.* 2012). In veterinary medicine, the aims of preliminary studies using the ARFI imaging have been to detect mammary tumours and to determine the elastographic characteristics of the normal liver, spleen and kidneys in dogs (Feliciano *et al.* 2014, Holdsworth *et al.* 2014).

The diagnosis of splenic malformations is of particular interest because this organ is involved in lymphatic, immune, circulatory and haematopoietic functions (Fry & McGavin 2007). The canine spleen is one of the main organs prone to the development of primary tumours, metastasis and a broad range of diseases of the haematopoietic system (Morris & Dobson 2007). To date, a conclusive diagnosis of various splenic pathologies is obtained mainly through the histopathological evaluation of samples collected via an ultrasound-guided biopsy or after splenectomy (Rodaski & Piekarczyk 2009). The first study evaluating shear velocity (quantitative evaluation) of the splenic parenchyma (at two depths: 0 to 2 cm, 2 to 4 cm) in adult dogs and assessing the effect of factors such as weight, depth and sex on the values on spleen elastography was performed by Holdsworth *et al.* (2014). However, the quantitative elastographic attributes of different splenic regions (head, body and tail) in dogs of various ages as well as the standardisation of the qualitative ARFI imaging of the canine spleen have yet to be performed.

Considering the applicability of ARFI elastography in human medicine and the paucity of studies on this imaging technique in small animals, the aims of this study were to employ ARFI elastography to evaluate the stiffness of the splenic parenchyma in healthy dogs from different age groups, and to determine the yet incompletely defined quality and quantity (shear velocity) standards of the technique. An additional aim was to compare the shear velocities to the quantitative image attributes obtained by the computerised image analyses of splenic ultrasonograms in order to determine the relative suitability of both methods for monitoring the possible changes in splenic parenchymal rigidity.

MATERIALS AND METHODS

The study was approved by the Animal Ethics and Welfare Committee of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Campus Jaboticabal, Brazil (protocol no. 11852/14). Thirty crossbred male dogs weighing 2 to 10 kg were selected for this study. The animals were deemed healthy based on the results of routine physical examination and complete blood analysis (including biochemical profile), urinalysis and a urinary protein creatinine ratio (UPC) evaluation. The dogs were then divided into three different groups: Group 1 ($n=11$), young animals: 3 months to 1 year of age (mean age = 6.0 ± 3.3 months and mean weight = 3.3 ± 1.0 kg); Group 2 ($n=17$), adults: 1 to 7 years of age (mean age = 3.1 ± 1.6 years and mean weight = 6.0 ± 1.8 kg); and Group 3 ($n=5$), old animals: >7 years of age (mean age = 9.4 ± 2.2 years and mean weight = 8.0 ± 1.6 kg).

B-mode ultrasonography

The hair covering the abdominal area was amply clipped to facilitate transcutaneous ultrasonographic examinations. Before each ultrasonographic examination, hydrophilic gel was applied to the abdominal skin and the surface of the probe to ensure appropriate contact. No sedation was required throughout the entire duration of scanning. All ultrasonographic examinations were performed by the same experienced operator (MARF). The B-mode ultrasonography utilised a 9.0-MHz linear-array transducer connected to the ACUSON S2000/SIEMENS diagnostic system (Siemens, Munich, Germany). The echotexture (homogeneous or heterogeneous) and echogenicity (hypo, hyperechoic or mixed) of the splenic parenchyma (relative to the renal cortex) and the size (increased, decreased or normal) and edges (triangular or normal) of the spleen recorded during the ultrasonographic scanning were all subjectively evaluated.

ARFI elastography

The ARFI elastography of the canine spleens also utilised a 9.0-MHz linear-array transducer (Feliciano *et al.* 2014), and the analysis of the elastographic parameters was accomplished with the commercially available software specifically designed for the qualitative and quantitative image analyses (Virtual Touch Tissue Quantification, Siemens, Germany). Immediately following the B-mode ultrasonography, greyscale elastographic (ARFI) images of the head, body and tail of the spleen were obtained. The images were assessed for the presence of deformities (i.e. the presence of white areas indicative of more elastic/less rigid/softer or more deformable regions) and dark areas (more rigid, harder, and not deformable). The quantitative elastographic evaluation entailed the determination of the shear velocity; an appropriate function on the diagnostic system was activated, and the calliper was placed within the splenic parenchyma. Six measurements in each position (head, body and tail) with a depth ranging from 0.5 to 2.0 cm were obtained and used to calculate the mean shear velocities (Fig 1). A calliper size was adjusted to ascertain that sufficient portions of the spleen that encapsulated the calliper entirely were evaluated for each splenic region (Fig 2).

B-mode ultrasonograms of the spleen containing the largest cross-sectional area of the organ were saved as still digital (*.jpeg) images. Computer-assisted image analyses of the splenic parenchyma were conducted using a commercially available image analytical programme (Image ProPlus®, Media Cybernetics Inc., San Diego, CA, USA). A polygon meter tool was used to compute the mean numerical pixel values (NPVs), pixel heterogeneity



FIG 1. An ultrasonographic image of the canine spleen subjected to quantitative acoustic radiation force impulse (ARFI) evaluation of the caudal portion (tail)

(standard deviation of NPVs) as well minimal and maximal pixel values from each area of interest (Fig 3).

The data were analysed with the PROC MEANS-SASTM (SAS Institute Inc., Cary, NC, USA) and Graph-Pad Prisma

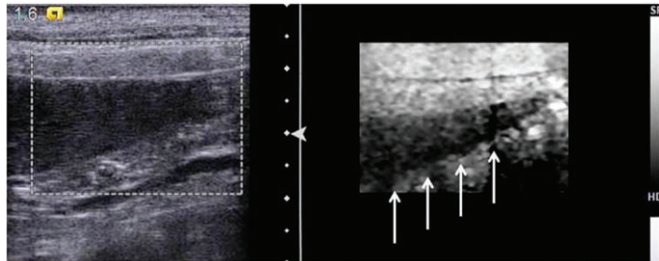


FIG 2. An ultrasonographic image of the canine spleen subjected to quantitative Acoustic Radiation Force Impulse (ARFI) evaluation (A) a B-mode image of the tail of the spleen; (B) elastographic analysis of the spleen (arrows)

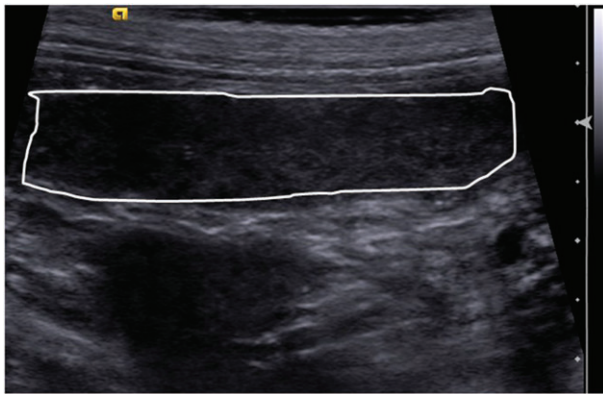


FIG 3. Diagram illustrating a placement of the polygonal meter used for determining the quantitative ultrasonographic attributes of B-mode splenic images using the ImageProPlus[®] analytical software

4TM statistical software (GraphPad Software Inc., La Jolla, CA, USA). Normality distributions and homogeneity of variances of the parameters and residuals were analysed by the Shapiro and F tests, respectively. The significance level was set at 95% for all tests performed. The raw or transformed averages for the shear velocities and quantitative echotextural variables of the different splenic segments were evaluated by a one-way analysis of variance (ANOVA). Correlations among mean shear velocities (averaged for the entire spleen) and quantitative echotextural characteristics of the spleen were assessed by the simple linear regression. All non-parametric data (i.e. echotexture, echogenicity, size and appearance of the edges as well as the detection of deformities in greyscale splenic ultrasonograms) were noted for each animal.

RESULTS

In the animals of this study, all of the spleens examined with the B-mode ultrasonography displayed a homogeneous echotexture, normal size and fine/normal borderlines. No parenchymal abnormalities (i.e., hyperechoic regions relative to the renal cortex) were detected in this study. The qualitative and quantitative elastography of the splenic head, body and tail in dogs could be performed without difficulty. On the basis of the qualitative elastography, the evaluated splenic parenchyma was not deformable and appeared homogeneously dark (Fig 1). Using the quantitative ARFI imaging, the mean shear velocity values were 2.32 m/s for the head, 2.16 m/s for the body, and 2.25 m/s for the tail of the spleen (Table 1; $P>0.05$). Mean shear velocity values did not vary ($P>0.05$) among animals allocated to the three age groups.

There were no significant ($P>0.05$) differences between the different age groups of dogs for the quantitative echotextural characteristics of the splenic ultrasonographic images (Table 2); the differences in minimal pixel values between the three groups

Table 1. Mean values ($\pm$ standard deviation) and 95% confidence intervals (CI 95%) for shear velocities of the canine spleen (tail, body and head) determined using the acoustic radiation force impulse (ARFI) quantitative elastography

Group/splenic segment	Tail	Body	Head	P value
Group 1 (n=11)	2.31 $\pm$ 0.33	2.09 $\pm$ 0.32	2.38 $\pm$ 0.58	0.24
(CI 95%)	2.10 to 2.53	1.88 to 2.30	1.99 to 2.77	
Group 2 (n=17)	2.32 $\pm$ 0.56	2.25 $\pm$ 0.63	2.34 $\pm$ 0.56	0.92
(CI 95%)	1.98 to 2.67	1.86 to 2.63	1.99 to 2.68	
Group 3 (n=5)	1.98 $\pm$ 0.27	2.10 $\pm$ 0.27	2.19 $\pm$ 0.36	0.51
(CI 95%)	1.69 to 2.27	1.81 to 2.38	1.8 to 2.57	
Overall (n=33)	2.25 $\pm$ 0.44	2.16 $\pm$ 0.47	2.32 $\pm$ 0.52	0.40
(CI 95%)	2.09 to 2.42	1.98 to 2.33	2.13 to 2.52	

Group 1, young animals – 3 months to 1 year of age; Group 2, adults – 1 to 7 years of age; Group 3, old animals – >7 years of age

Table 2. Mean values ($\pm$ standard deviation) for the splenic echotextural parameters determined with the Image ProPlus[®] analytical software

Variables/group	Group 1	Group 2	Group 3	P value
NPVs	38.17 $\pm$ 12.28	32.18 $\pm$ 15.16	30.95 $\pm$ 9.02	0.46
MaxPV	171.8 $\pm$ 50.86	218.5 $\pm$ 61.69	190.3 $\pm$ 62.43	0.16
MinPV	2.38 $\pm$ 0.58	2.34 $\pm$ 0.56	2.19 $\pm$ 0.36	0.08
PSD	19.50 $\pm$ 10.40	28.93 $\pm$ 11.18	27.89 $\pm$ 13.73	0.19

Numerical pixel values (NPVs), maximal pixel value (MaxPV), minimal pixel value (MinPV), and standard deviation of mean pixel values (or pixel heterogeneity – PSD). Group 1, young animals – 3 months to 1 year of age; Group 2, adults – 1 to 7 years of age; Group 3, old animals – >7 years of age

of dogs was not significant ($P=0.08$). No significant correlations were recorded between mean shear velocities and echotextural parameters studied ($P>0.05$; Table 3).

DISCUSSION

The splenic elastography in the dogs of this study could consistently be performed without difficulty. In contrast to a previous study by Holdsworth *et al.* (2014) looking at the application of splenic elastography in dogs, the present investigation provided reference values for elastographic parameters of healthy canine spleens. To the best of the authors' knowledge, this is the first report of the elastographic characteristics of the various anatomical regions of splenic parenchyma in clinically healthy dogs (based on a broad range of clinical tests including serum biochemistry) of various ages. In addition, the results of the ARFI imaging were compared with those obtained using the computerized assessment of greyscale ultrasonograms of the dogs' spleen.

Variations in the size and functional characteristics of the spleen between young and old dogs were the main reason for including the three age groups of animals in this study. Young animals tend to have larger spleens than older individuals probably due to decreased haematopoietic reserve in older dogs (Grandmaison *et al.* 2001).

The B-mode ultrasonography is the most frequently used imaging modality for the evaluation of the spleen in dogs because it is highly effective in detecting macroscopic lesions in the splenic parenchyma, and for evaluating the size and location of the organ (i.e. detection of splenomegaly or splenic masses) (Kisseberth & McEntee 2008, Bandinelli *et al.* 2011). However, this technique is neither sufficiently sensitive to identify diffuse pathological changes, or specific to distinguish between malignant and benign pathologies (Vescoci *et al.* 2009, Garcia *et al.* 2012). Up until now a definitive diagnosis could only be obtained using histopathological analysis of the lesions (Rodaski & Piekarczyk 2009). The qualitative and quantitative elastographic parameters described in this study constitute the reference values that can be employed in subsequent studies of the applicability of the ARFI imaging for detection of a vast range of splenic lesions in dogs.

The alterations in the shear velocity of the splenic tissue have proven to be very useful in detecting and diagnosing chronic liver conditions (cirrhosis), portal hypertension (Bota *et al.* 2010) and certain vascular alterations (oesophageal varices) (Ye *et al.* 2012) in humans. However, there appear to be no reports on the clinical

application of ARFI elastography for detecting and diagnosing canine splenic disorders. The only preliminary results obtained thus far in dogs involved the evaluation of mammary neoplasms using the ARFI method (rigid and non-deformable mammary neoplasms and high shear velocities are indicative of malignancy; Feliciano *et al.* 2014). Novel data obtained in this study may therefore pave the way to a more widespread use of non-invasive imaging techniques for the detection of splenic abnormalities in veterinary practice.

In this study, the splenic parenchyma consistently exhibited as rigid and homogeneous tissue that was not easily deformable, suggesting that qualitative elastography could potentially be performed to locate the altered intra-parenchymal areas (hard/soft or deformable/not deformable). This specific evaluation may help to detect both focal areas and diffuse histopathological changes in the canine spleen. In general, the sonographic parameters (echogenicity and echotextural changes) of the internal organs and tissues determined by B-mode ultrasonography are not associated with the malignancy of the lesion observed in ultrasonographic examination (Garcia *et al.* 2012). In this study, it has been demonstrated that the quantitative ultrasonographic variables obtained with the computerised analysis of digital ultrasonograms were not correlated with the quantitative elastographic parameters of the canine spleens. However, the absence of the quantitative correlations in this study does not preclude the existence of such relationships in animals with splenic disease; further studies are needed to clarify this. Because the increased tissue stiffness is directly related to the malignancy of the lesion, it has been suggested that a rise in overall tissue heterogeneity and the occurrence of areas with greater rigidity or stiffness/increased shear velocity (compared to the healthy tissue) may be indicative of the presence of a mammary neoplasm (Tozaki *et al.* 2011, Bai *et al.* 2012, Feliciano *et al.* 2014). It is therefore logical to assume that any changes in tissue stiffness observed during the course of splenic diseases in dogs may be effectively monitored with ARFI imaging, increasing the accuracy of the ultrasonographic technique for detecting an array of splenic disorders in dogs.

Mean shear velocities of the canine spleen did not vary between different splenic regions in this study and they were similar to those recorded in healthy humans (2.44 m/s for the hilum, 2.40 m/s for the pole region, and 2.04 ± 0.28 m/s in all of the splenic parenchyma; Bota *et al.* 2010, Gallotti *et al.* 2010) and to the maximum values for this variable found in adult dogs (an interquartile range of 1.59 to 2.4 m/s at 0 to 2 cm and 1.45 to 1.94 m/s at 2 to 4 cm depth; Holdsworth *et al.* 2014). It is, however, important to note that even though in the dogs of the present study the measurements of shear velocities were taken from the three different regions of the spleen and from animals of different ages, observations were restricted to depths less than 0.5 to 2.0 cm).

In their earlier study, Holdsworth *et al.* (2014) observed the influence of some conditions on the splenic shear velocity in dogs; there was an increase of 0.021 m/s for every 1 kg increase in body weight and a decline by 0.197 m/s for every 1 cm increase in depth. Furthermore, the splenic shear wave velocity values in males were on the average 0.529 m/s lower compared to those in females. To minimise the potential confounding effects of sex and body conformation (weight, abdominal volume and scan-

Table 3. R (coefficient of correlation) and P values for correlations among the splenic shear velocity and quantitative echotextural variables

Parameters	R	P value
MSV×NPVs	-0.0930	0.63
MSV×MaxPV	0.0994	0.61
MSV×MinPV	0.241	0.21
MSV×PSD	0.279	0.14

MSV, mean shear velocities; NPVs, numerical pixel values; MaxPV, maximum pixel value; MinPV, minimum pixel value; standard deviation of mean pixel values (or pixel heterogeneity - PSD)

ning depth) on splenic elastography results, only crossbred males with little variation in body weight and size were used. In the ensuing studies, however, all of the influencing factors including the animals' age, splenic region/segment and scanning depth would have to be considered as certain focal splenic lesions may be larger than 2 cm in size and/or may be located below the 2-cm scanning range. As noted by Holdsworth *et al.* (2014) in their previous report, it is also necessary to conduct a study with a larger number of animals and to evaluate dogs of different breeds and sex to examine the interactions of these factors on the elastographic and echotextural characteristics of the canine spleen.

It should also be mentioned that the quantitative values obtained using the ARFI elastography of the spleen in healthy dogs are important reference values that can be used in studies of focal splenic abnormalities, and may potentially assist in the detection of chronic liver diseases and circulatory abnormalities in dogs and other animals in the same manner as they are used in humans (splenic shear velocities of 3.65 ± 0.32 m/s in human patients with portal hypertension (Gao *et al.* 2012); 3.28 ± 0.45 m/s in humans with a history of variceal bleeding and 3.19 ± 0.55 m/s in humans with oesophageal varices (Bota *et al.* 2010)).

In conclusion, this study provides novel information on the splenic elastographic parameters in clinically healthy dogs, providing quantitative values for tissue echogenicity, qualitative and quantitative findings of ARFI elastography, and an evaluation of different regions of the canine spleen in animals of various ages. In particular, the quantitative and qualitative ARFI elastography of the spleen in dogs could be easily performed and may provide additional valuable criteria for the evaluation and diagnosis of splenic abnormalities, typically accomplished in veterinary medicine with the use of the B-mode ultrasonography.

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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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