



## ROLE OF STRAIN ELASTOGRAPHY IN DIFFERENTIATING MALIGNANT HYPOECHOIC SPLENIC LESIONS IN DOGS: PRELIMINARY RESULTS

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### Summary

Barella, G., M. Lodi & S. Faverzani, 2016. Role of strain elastography in differentiating malignant hypoechoic splenic lesions in dogs: Preliminary results. *Bulg. J. Vet. Med.* (online first).

This study aims to assess the repeatability and reproducibility of strain elastography in the evaluation of splenic nodules and to verify if it can differentiate benign from malignant splenic lesions in dogs. Twenty-four dogs with a single splenic hypoechoic lesion, underwent strain elastosonography. The strain ratio (SR) and hardness value (HV) were calculated. Repeatability and reproducibility were assessed by the coefficient of variability (CV) and the K of Cohen respectively. ANOVA and Fisher exact test verified the differences between the SR values and HV of benign versus malignant lesions. The odds ratio was also calculated. The SR and HV of benign lesions were different from those of malignant lesions ( $P < 0.05$ ). Malignant lesions tended to have a  $SR \geq 1.5$  ( $P = 0.0003$ ) and an  $HV \geq 70\%$  ( $P = 0.001$ ). The correlation between  $SR \geq 1.5$  and malignancy was significant (OR, 80.6;  $P = 0.0067$ ) as the one between  $HV \geq 70\%$  and malignancy (OR, 47.22;  $P = 0.013$ ). This technique was repeatable and reproducible (CV  $0.08 \pm 0.03$  and  $K = 1$  for the SR; CV  $0.08 \pm 0.05$  and  $K = 0.66$  for the HV). Elastosonography can differentiate malignant from benign canine hypoechoic splenic lesions.

**Key words:** spleen, splenic lesions, strain elastography, ultrasound

### INTRODUCTION

Focal splenic lesions are alterations of the parenchyma that make the organ non-homogeneous and can cause a deformation of its profile. Ultrasound can easily detect splenic lesions in dogs regardless of their clinical condition. Usually for big lesions or lesions with a high risk of rupture, an elective splenectomy is strongly suggested, while small lesions remain a diagnostic challenge. To our knowledge

hyperechoic lesions are mostly benign while the not-hyperechoic lesions can be benign or malignant. Unfortunately, the B-mode appearance of hypoechoic splenic lesions relates to any specific disease. Even the colour and power Doppler evaluation of blood flow to the spleen masses does not provide a reliable differentiation between benignity and malignancy (Sharpley *et al.*, 2012). Moreover, the contrast

enhanced ultrasound has been defined as a limited screening diagnostic method and its role is questionable for splenic pathology (Ohlerth *et al.*, 2008; Ivancic *et al.*, 2009; Taeymans & Penninck, 2011). Only the presence of tortuous feeding vessels, throughout all perfusion phases, may improve the accuracy in characterising benign splenic lesions versus malignant (Taeymans & Penninck, 2011). The fine needle aspiration can be considered as the first step towards an accurate diagnosis representing a low risk procedure that can be performed without sedation. This technique presents a high sensibility for the diagnosis of extramedullary haematopoiesis or round cells neoplasia but is less sensible for connective tissue tumours that tends to exfoliate cells poorly (Hayes & Ladlow, 2012). However in some cases the location or the appearance (high risk of rupture) of the lesions makes it very difficult if not impossible the ultrasound-guided percutaneous sampling. Sedation or general anesthesia for uncooperative patients are often required (Holdsworth *et al.*, 2014); however this procedure can be not viable in case of critical patients. Needle core biopsy has been proposed as a complementary method but the requirement of sedation, the costs and the possibility of major complications made the recurrence of this procedure questionable (Taeymans & Penninck, 2011). Real-time or strain elastography is a non-invasive technique that can be used in medicine to assess tissue stiffness or tissue displacement in response to an applied force. The deforming force applied to the tissue derives from an external compressor (e.g. perpendicular movements of the ultrasound transducer) or physiologic functions (e.g. breathing, cardiac movements) (White *et al.*, 2014). The usefulness of this method is based on the fact that

pathological changes in tissues also affect in their stiffness (neoplasia tends to be harder than normal tissue due to high cell density). Differences in tissue displacement (called strain) are calculated and presented as a colour map (elastogram) that overlaps the B-mode image. The colour map documents the relative elasticity of the tissues included in the region of interest expressed in different colours. The deformability of the organ (elasticity) depends on the tissue composition; neoplastic tissues have higher cell density and therefore a reduced elasticity (increased tissue stiffness) (Goddi *et al.*, 2012). Similarly, fluids, physically, cannot be compressed and an increased blood flow in an organ increases its hardness (Hirooka *et al.*, 2011). In human medicine, malignant parenchymal lesions are significantly stiffer than surrounding normal parenchyma (Wells & Liang, 2011; Barr, 2012; Teng *et al.*, 2012; Wang *et al.*, 2013). Strain elastography has proven to be a feasible technique for estimating tissue stiffness in the healthy canine liver, spleen, kidneys and prostate (Jeon *et al.*, 2015). To our knowledge there are no studies concerning the evaluation of splenic lesions using real time elastosonography neither in humans nor in small animal medicine. The aim of this study is to assess the repeatability and reproducibility of real time elastography in the evaluation of splenic lesions in dogs and to verify if this technique can differentiate benign from malignant lesions.

## MATERIALS AND METHODS

Medical records belonging to dogs referred to our hospital, between January 2014 and January 2015, were evaluated in order to select all patients presented for abdominal ultrasound having a solitary

hypochoic splenic lesion as the only significant finding. All the ultrasonographic examinations were performed using an Esaote MyLab 70 (Genova, Italy) equipped with a multifrequency linear transducer (7.5–13 MHz) in association with a Strain Elastography module. Dogs were evaluated, after hair clipping and ultrasonographic gel applied, in right lateral recumbency in order to have the left part of the abdomen upward. Dogs underwent B-mode evaluation of the entire abdomen and elastosonographic evaluation of the spleen. The left intercostal approach was used to evaluate in longitudinal section the head of the spleen; the splenic body and tail were scanned throughout sagittal and transverse planes from the ventral and lateral abdominal wall. Each focal splenic lesion recorded in B-mode was measured in both diameters and only lesions of a maximum 4 cm width were selected. Only splenic lesions located at a maximum depth of 4 cm and not close to the visceral margins of the spleen were considered. Strain elastography was used to assess the qualitative and relative stiffness of splenic lesions that were comprised in a selected region of interest (ROI). The selected ROI comprised all the sonographic field of view (a maximum of 5 cm depth) and included the entire splenic lesion and a same sized portion of normal splenic parenchyma almost located at the same depth. Stomach and great abdominal vessels were not considered in the ROI. The Real-time elastosonographic examination was performed in succession by 2 operators maintaining the same parameters such as: acoustic window, depth, ROI size, gains and frequency. Tissue compression was performed, in order to obtain the elastogram of each lesion, using the ultrasonographic probe. A grey spiral located on the screen that turned into green indicated

the adequate amount of pressure. Images were acquired after at least 5 seconds lingering of the green spiral. In the elastogram, hard areas (areas of low strain) were coded in blue, intermediate stiffness areas were coded in green and soft areas were coded in red (areas of high strain).

Each operator obtained 5 images of each splenic lesion and on these images 2 measurements were performed. The strain ratio (SR or  $z2/z1$ ; where  $z1$  represented the entire lesion and  $z2$  represented a same size portion of normal splenic parenchyma located at the same depth) and the hardness value (HV; percentage of areas in the lesion coded as hard) were calculated for each lesion. The mean and standard deviations of all measurements performed by the two operators were calculated. All splenic lesions were subjected to ultrasound guided fine needle biopsy (“not aspiration” technique using a 22–23 Gauge needle) and/or histologic evaluation (performed after splenectomy). The coefficient of variability (CV) and the K of Cohen were calculated in order to assess the inter-observer and the intra-observer variability of this method respectively. The ANOVA and the Fisher exact test were used to verify the differences between the SR values and HV of benign versus malignant lesions. The cut-off value for the Fisher test was set at 1.5 for SR and at 70% for the HV according to a ROC curve analysis. The odds ratio (OR) was also calculated considering: malignancy as bad outcome and dogs with values of  $HV > 70\%$  and  $SR > 1.5$  in the exposed group. A  $P < 0.05$  was considered significant. The sensibility (Se), the specificity (Sp), the positive predictive value (PPV), the negative predicting value (NPV), the positive likelihood ratio (PLR) and the negative likelihood ratio (NLR) were also calculated. Positive and nega-

tive likelihood ratios were calculated to determine the diagnostic value of strain elastography in differentiating splenic lesions in the two groups. Likelihood ratios provide a statistical method for assessing the clinical usefulness of a diagnostic test for ruling out (NLR<0.25) and ruling in (PLR>4) a disease (Wall *et al.*, 2015). To avoid extreme calculated values, the results with zero false positive or false negative were modified by adding 1 subject into each cell of the 2×2 table (Lamb & Nelson; 2015).

RESULTS

Twenty-four dogs met the inclusion criteria: 2 Beagles, 1 Border Collie, 1 Boston Terrier, 5 English Cocker Spaniels, 1 English Foxhound, 1 Flat Coated Retriever, 1 French Bulldog, 2 German Shepherds, 1 Golden Retriever, 1 Hovawart, 1 Italian Spinone, 2 Labrador Retrievers, 4 Mixed Breeds, 1 Shitzu. The mean age of patients was 10.44 ± 3.2 years; 11 dogs were males (3 neutered) while 13 were females (12 neutered). Mean values of minor and major diameters of lesions were respectively; 1.35±0.94 cm and 1.63±0.96 cm. The biggest lesion measured 3.6×3.9 cm while the smallest 0.49×0.5 cm. Sixteen of 24 splenic lesions were benign (67%; 2 extramedullary haematopoiesis, 14 nodular hyperplasia) while 8 of 24 were malignant (33%; 6 haemangiosarcoma, 2 lymphoma). All dogs underwent fine needle

biopsy followed in 6 cases by histology after splenectomy. The benignity of lesions was also confirmed in seven dogs presenting nodular hyperplasia followed and re-evaluated (B-mode and real time elastography) from 3 to 17 months after the first ultrasonographic exam. None of these patients presented any changes concerning the B-mode (dimension and echotexture) and elastosonographic (SR and HV) appearance of splenic nodules. All these patients underwent a second fine needle biopsy of the lesion that showed the same diagnosis. No differences were observed between benign and malignant lesions considering: lesions size, canine sex and age (P>0.05).

Considering a cut-off value of 1.5 for SR, significant differences were observed between benign (mostly soft) and malignant (mostly hard) lesions (P=0.0009; Se 70%, Sp 94,1%, PLR 11.9, NLR 0.32, PPV 87.5%, NPV 84.2%, Table 1). No benign lesions presented a SR value ≥1.5 while 6 malignant lesions presented a SR value ≥1.5 and 2 malignant lesions <1.5. (Table 2). In one case, due to poor image quality, the SR could not be calculated.

Considering a cut-off value of 70% for HR significant differences were observed between benign (mostly soft) and malignant (mostly hard) lesions (P=0.0044; Se 90%, Sp 72,22%, PLR 3.24, NLR 0.14, PPV 64.3%, NPV 92.9%, Table 1). No malignant lesions had a HV <70% while 12 benign lesions presented a HV<70% and 4 benign lesions ≥70% (Table 2).

**Table 1.** Values of positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV), specificity (Sp), sensitivity (Se), odds ratio (OR) and statistical significance (p) of strain ratio and hardness value measurements

	PLR	NLR	PPV	NPV	Sp	Se	OR	P value
Strain ratio	11.9	0.3	87.5	84.2	94.1	70	80.6	0.0067
Hardness value	3.2	0.14	64.3	92.9	72.2	90	47.3	0.013

**Table 2.** Distribution of splenic lesions according to strain ratio values and hardness values and diagnosis (Fisher exact test)

Strain ratio	<1.5	≥1.5	Hardness Value	<70%	≥70%
Benign lesions	15	0	Benign lesions	12	4
Malignant lesions	2	6	Malignant lesions	0	8

Data concerning individual strain ratios and hardness values are reported in Table 3.

The CV values were  $0.08 \pm 0.03$  for the SR and  $0.08 \pm 0.05$  for the HV.

The K of Cohen showed a perfect agreement between operators concerning the HV ( $k=1$ ) and a good agreement concerning the SR ( $k=0.66$ ).

The OR showed that the correlation between  $SR \geq 1.5$  and malignancy was statistically significant (OR, 80.6;  $P=0.0067$ ) as the one between  $HV \geq 70\%$  and malignancy (OR, 47.22;  $P=0.013$ ).

## DISCUSSION

The prevalence of benign versus malignant lesions in our sample (frequency respectively of 67% and 33%) is consistent with other studies about the same subject (Rossi *et al.*, 2008; Christensen *et al.*, 2009; Watson *et al.*, 2011; Cole *et al.*, 2012; Hayes & Ladlow, 2012). Nodular hyperplasia was the most represented lesion (13 of 24 lesions, 54%), while haemangiosarcoma was the most represented between malignant lesions (6 of 8, 75%). These results are also similar to those reported in other papers (Christensen *et al.*, 2009; Watson *et al.*, 2011; Jun-Yong *et al.*, 2012). We observed that benign lesions tend to be as soft as the surrounding normal splenic parenchyma, while malignant lesions tend to be harder ( $P<0.05$ ) (Fig. 1). In our opinion, the hardness of malignant lesions can be ex-

plained considering their high cells density, or in case of haemangiosarcoma their areas containing fluids (incompressible for physics). All malignant lesions presented  $HV \geq 70\%$  and 6 of 8 presented  $SR \geq 1.5$ . The 2 malignant lesions having  $SR < 1.5$  were one lymphoma and one haemangiosarcoma and we can hypothesise that the necrotic and haemorrhagic areas in these tumours made the lesions softer and more deformable. All benign lesions presented a  $SR < 1.5$  and 12 of 16 presented a  $HV < 70\%$ . The four of 16 benign lesions resulting harder than normal splenic parenchyma ( $HV \geq 70\%$ ) were nodules of hyperplasia; these lesions may have many vessels containing, obviously, fluids that are incompressible and in our opinion made the lesion harder. Considering the HV our tests showed a high Se (90%) and a quite low Sp (72.2%); while considering SR a high Sp (94.1%) and a quite low Se (70%). In our opinion these results can be due to the retrospective nature of our work that can contain some BIAS. However the value of NLR  $< 0.25$  concerning HV and the value of PLR  $> 4$  concerning SR hearten us and let us assert that this values can be useful in order to respectively rule out and rule in the disease.

Considering the strain ratio between the stiffness of the spleen and the stiffness of the abdominal wall, the inter- and intra-observer variability of strain elastography have already been demonstrated (Jeon *et al.*, 2015). Our study showed the repeata-

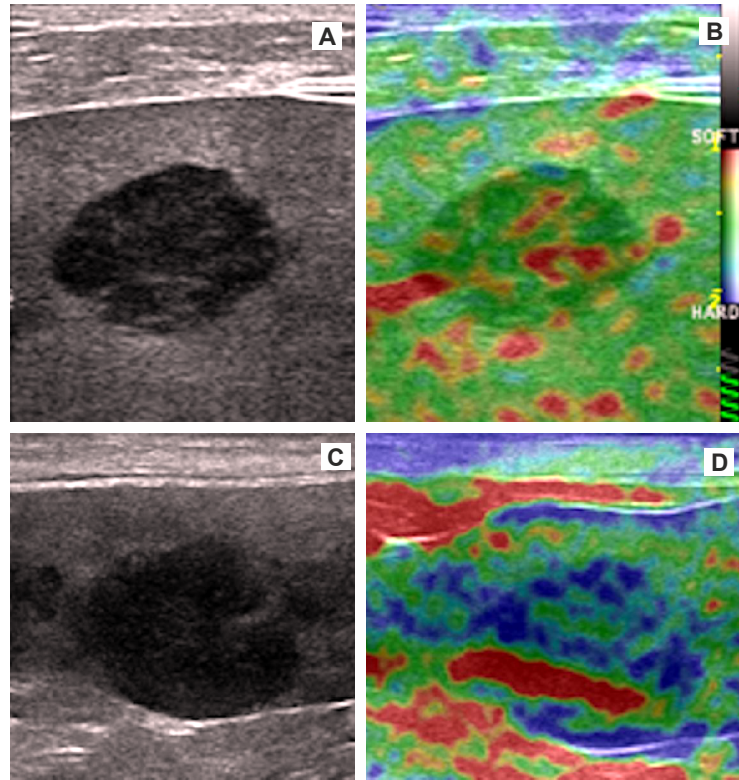
bility and reproducibility of strain ratio between the normal and altered splenic parenchyma. We obtained a CV <10%

(inter-observer variability; considering both value of SR and HV), that could be related to inherent variability of the technique as it requires repeated movements

**Table 3.** Strain ratio and hardness values of splenic lesions

Dog	Strain ratio	SD	Hardness value	SD	Diagnosis
1	0.86	0.1	47.84	0.1	Extramedullary haematopoiesis
2	1.07	0.11	32.72	8.99	Extramedullary haematopoiesis
3	1.11	0.13	62.73	4.01	Nodular hyperplasia
4	1.45	0.07	69.65	0.65	Nodular hyperplasia
5	NC	NC	63	14.76	Nodular hyperplasia
6	0.95	0.04	32.47	1.82	Nodular hyperplasia
7	0.96	0.03	44	2	Nodular hyperplasia
8	0.87	0.03	19	4	Nodular hyperplasia
9	1.26	0.14	90.75	7.24	Nodular hyperplasia
10	0.93	0.1	37.55	12.54	Nodular hyperplasia
11	1.19	0.07	82.29	7.83	Nodular hyperplasia
12	1.3	0.15	83.76	9.16	Nodular hyperplasia
13	1.21	0.11	53.7	8.5	Nodular hyperplasia
14	1.11	0.11	69.87	16.14	Nodular hyperplasia
15	1.06	0.21	65.49	11.9	Nodular hyperplasia
16	1.26	0.08	98.44	1.29	Nodular hyperplasia
17	2.56	0.23	77.43	1.93	Haemangiosarcoma
18	1.59	0.25	84.97	4.75	Haemangiosarcoma
19	2.72	0.06	93.87	5	Haemangiosarcoma
20	1.08	0.27	80.97	13.61	Haemangiosarcoma
21	2.25	0.27	93.23	3.46	Haemangiosarcoma
22	2	0.54	99.97	0.07	Haemangiosarcoma
23	0.91	0.09	85.34	7.62	Lymphoma
24	1.7	0.09	91.21	2.54	Lymphoma

SD: standard deviation.



**Fig. 1.** A-B: B-mode and strain elastography image of a benign splenic lesion (extramedullary haematopoiesis) presenting almost the same stiffness of the surrounding splenic parenchyma. C-D: B-mode and strain elastography image of a malignant splenic lesion (haemangiosarcoma) coded as harder than the contiguous splenic parenchyma.

of compression and retraction of the probe as equal as possible between them. Concerning the intra-observer variability, we obtained an overall very good agreement between operators considering both SR and HV. In our opinion, the variability observed could depend on the different force applied by the two operators with the probe. This represent a technical problem of strain elastography hard to overcome, as reported in literature (Mulligan *et al.*, 2015). Considering OR we observed that a lesion presenting a  $SR \geq 1.5$  or a  $HV \geq 70\%$  has respectively almost 80 and 47 times higher possibility to be malignant than one with  $SR < 1.5$  or  $HV < 70\%$ .

There are some important limitations in this study. The work was retrospective and a small number of animals with a little lesions variation was considered. However, this work represents an initial approach to the study of splenic lesions in dogs using stain elastography. Further studies should also investigate the role of strain elastography in the monitoring of that benign lesions that with time can turn into malignant and change their stiffness; or in the monitoring of those lesions that we cannot sample due to difficult situations.

In conclusion, real time elastography seems to be useful in differentiating ma-

lignant from benign splenic hypoechoic lesions less than 4 cm width. Lesions with  $SR \geq 1.5$  have a high probability to be malignant while lesions with  $HV < 70\%$  have a high probability to be benign. However, the right way to reach a diagnosis cannot leave aside cytological and/or histological evaluation.

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Paper received 01.04.2016; accepted for publication 10.06.2016

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