11 Strain elastography for the assessment of skin nodules in
12 dogs: preliminary results

13

14 Giulia Brizzi[1], Paola Crepaldi[2], Paola Roccabianca[3], Eric Zini[4,5,6], Simona
15 Morabito[7], Edoardo Auriemma[4], Giordana Zanna[4]

16
17 1 Ambulatorio Veterinario Brizzi, Corso Matteotti 17, 89044, Locri, Italy
18 2 Department of Agricultural and Environmental Sciences Production, Landscape, Agroenergy, University of
19 Milan, Via Celoria 2, 20133, Milan Italy
20 3 Department of Pathology, Hygiene and Veterinary Public Health, Università degli studi di Milano, Via
21 dell'Università 6, Lodi 26900, Italy
22 4 Istituto Veterinario di Novara, Strada Provinciale 9, 28060, Granozzo con Monticello, NO, Italy.
23 5 The Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260,
24 8057, Zurich, Switzerland.
25 6 Department of Animal Medicine Production and Health, Università degli Studi di Padova, Viale dell'Università
26 16, 35020, Legnaro, PD, Italy.
27 7 Animal Health Trust, Lanwades Park, Kentford, Newmarket Suffolk CB8 7UU, United Kingdom
28

29 Abstract

30 Background

31 Strain elastography (SE) is a modern imaging technology that provides an additional way of
32 evaluating the changes in soft tissue elasticity caused by pathophysiological processes.
33 Despite its widespread use in human medicine, only a few studies on the application of SE in
34 veterinary medicine are available.

35 Objectives

36 To evaluate the potential usefulness of SE as an integrative imaging model in the standard
37 ultrasound technique to better discriminate between inflammatory and neoplastic skin nodules
38 in dogs.

Animals

Fifty-one client-owned dogs with clinical evidence of single or multiple skin nodules detected during routine dermatological examination.

Methods and materials

Margins, echogenicity, echo-structure, calcification and vascularisation of 65 skin nodules were assessed with ultrasound, and SE was used to score qualitative (E-score, E-index, E2) and semiquantitative (SR) parameters. A comparison of diagnostic yields with cytological and histological findings as the gold standard was performed.

Results

Mast cell and benign follicular tumours showed the highest E-scores and SRs among neoplastic nodules; statistically significant differences were not detected. Calcific and nonvascularised nodules showed significantly higher E-index values than the others. Overall, a negative correlation was observed between the longitudinal diameter of skin nodules and the qualitative elastic parameters.

Conclusions and clinical importance

In this study, SE proved to be useful to identify only a subset of nodules such as mast cells and hair follicular tumours. Although evidence supporting the use of SE in evaluating skin nodules was demonstrated to below, indicators to guide further research were developed.

Introduction

Ultrasound elastography was first introduced in the 1970s but only in recent years it has proven effective in the qualitative and quantitative assessment of tissue viscoelastic properties in humans.[1-3] This technique takes advantage of the changes of soft tissue elasticity caused by pathophysiologic processes such as aging, inflammation and uncontrolled cell growth, and measures tissue displacement in response to a transient mechanical force applied to the skin surface.[4] The resulting mechanical response called displacement or strain, generates ultrasound waves that are collected and translated to the video screen in a two-dimensional color map called elastogram. An elastogram is characterized by a continuum of colors varying from red to green to blue, designating several degrees of tissue elasticity. Normally, low strain corresponding to stiff tissue is displayed in blue, whereas high strain corresponding to soft tissue is displayed in red. However, this chromatic scale can differ depending on the elastographic software.[5-7]
Nowadays, ultrasound elastography can be divided into a quasi-static or strain-based elastography (SE) and a dynamic or shear wave elastography (SWE) for which a constant manual force and a time-varying oscillatory force are applied to the tissue, respectively.[1-3] Despite SWE has strong advantages over SE, as it is more

reproducible and relies on automatic shear wave generation, in humans SE is to date the most widely available commercial technique with high diagnostic sensitivity and accuracy when utilized to discriminate malignant from benign lesions and has an important role in diagnosis, staging, treatment and follow-up of many skin disorders.[8-14.] In particular, SE allows a qualitative assessment of the examined tissue based on distribution of colors, visual point scales and quantifying hard areas.[1-7] In order to decrease the inter-observer variability, a semi-quantitative measurement called strain ratio (SR) that provides information on the relative stiffness of a target lesion in comparison with the surrounding healthy tissue is also used.[15]

Despite the widespread use in human medicine, few studies on the application of SE are available in veterinary medicine. For example, SE has been used to assess intra-abdominal organs as liver, prostate, testicles, renal parenchyma and malignant nodules of breast and spleen, and only in one study it has been promoted as a complementary tool for differentiating lipomatosus from malignant subcutaneous neoplasms in dogs.[16-21]

The aim of this study was two-fold. First, to evaluate the potential usefulness of SE to discriminate between inflammatory and neoplastic skin nodules in dogs using qualitative and semi-quantitative evaluations. Second, to identify elastic patterns in order to associate elastosonographic with histopathological findings and develop an integrative imaging model to the conventional ultrasound technique.

Material and methods

Study population

Fifty-one client-owned dogs with clinical evidence of single or multiple skin nodules detected during routine dermatological examination were included. Informed owner consent was obtained prior to any procedure.

Inclusion criteria and initial examination

Dogs of any breed, body weight, sex and body condition score were enrolled on the basis of the following inclusion criteria: (i) nodule's diameter between 0,5 and 5 cm in length; (ii) no Fine Needle Aspiration Cytology or Needle Core Biopsy before ultrasound examinations; (iii) no administration of systemic and topical glucocorticoids or other medication during the previous two weeks; (iv) no clinical evidence of severe dehydration (>5%); (v) for intact female dogs, not being pregnant or lactating; (vi) normal complete blood cell count and routine serum biochemical analysis.

Conventional ultrasound and strain elastography evaluations

Conventional ultrasound (B-mode) and strain elastography (SE) examinations were performed using the Logiq S8 imaging device (GE Healthcare, Milwaukee, Wisconsin) equipped with a multifrequency linear transducer (L11, 8,5-10 MHz) in

118 association with a strain elastography software (LogiQ S8 Strain Elastography
119 software, GE Healthcare, Milan, Italy). B-mode and SE imaging were performed at
120 the same time according to standard procedures.
121 Based on the locations of skin nodules, lateral, dorsal or sternal recumbency was
122 adopted. The area of interest was gently clipped, and a copious amount of acoustic
123 gel was used over the surface of the lesion and surrounding healthy skin to provide
124 adequate contact, avoiding alteration of shape or blood flow of the nodule. B-mode
125 ultrasound, with and without Doppler, was initially performed at 10MHz and images
126 were obtained in the longitudinal planes. Skin nodules with surrounding normal tissue
127 were imaged in a single field of view and their longest diameter was measured.
128 Margins were evaluated as well-defined or ill-defined, echo-structure as
129 homogeneous or heterogeneous, calcification as present or absent. Echogenicity
130 relative to adjacent normal tissue was classified as hypoechoic, hyperechoic,
131 isoechoic to the subcutaneous space. Power and color Doppler setting was also used
132 to detect the optimal visualization of vessels, and macro-vascularization was
133 categorized in absent or present.
134 Immediately after B-mode ultrasonography, the elastography investigation was
135 performed. Manual, low frequency, perpendicular oscillations, centered on the target
136 lesion, were applied to obtain tissue deformation. The region of interest (ROI) in the
137 strain image was enlarged to cover both the entire lesion and the surrounding normal
138 tissue. The force transmitted was appropriately calibrated according to a green spiral
139 bar on the monitor, and elastographic images were acquired. A dual screen mode
140 was used for simultaneous displaying both the B-mode and elastographic images.
141 Elastic properties were visualized on the monitor as a color-coded spectrum ranging
142 from blue to red, corresponding to a low strain (less deformable) or high strain (more
143 deformable). Nodules were scanned in longitudinal planes to obtain a hemi-section
144 corresponding to the best approximation of the slice that would be interpreted on
145 histopathology.
146 Both qualitative and semiquantitative evaluations were performed and novel and
147 more accurate elasticity parameters as E2 and SR were also applied. For the
148 qualitative evaluations, E-score, E-index and E2 values were recorded. E-score was
149 attributed according to the Alam score system adopted in human medicine for
150 discriminating benign from malignant cervical lymph-nodes.[22] Five elasticity patterns
151 were assigned by the operator based on intralesional distribution of colors. Score 1
152 was for absent or small hard area; score 2 for a hard area ≤ 45%; score 3 for a hard
153 area ≥ 45%; score 4 if the nodule had a peripheral hard area and a central soft area;
154 score 5 if the hard area occupied the entire lesion.
155 The amount of soft and hard area in the selected ROI drawn with the anatomical
156 guide of the nodule on the B-mode image, was quantified using a commercial
157 software (E-Index function, GE Healthcare, Milan, Italy). E-index values ranged from
158 0 to 6: a higher value indicated greater stiffness and a color closer to blue on the
159 elastogram. Finally, E2 corresponding to the E-index value of the area collected with

skin biopsy and interpreted by histopathology, was calculated. The semi-quantitative SR evaluations was computed between two similar ROIs drawn as large as possible and at similar depth on pathologic and healthy tissue with the use of E-Ratio function (E-Ratio function, GE Healthcare, Milan, Italy). A SR value >1 represented increased tissue stiffness in the skin nodule relative to the reference healthy tissue selected.

Cytological and histopathological diagnosis
After ultrasound was completed, nodules were sampled by fine needle aspiration cytology (FNAC) using a 22-gauge needle and 5 ml disposable plastic syringe. Air-dried and alcohol fixed smears were obtained. Cytological specimens were stained in May-Grunwald Giemsa® (Alcyon SpA, Cherasco, CN, Italy) and screened by a clinical pathologist.
A skin biopsy collected under local anesthesia using a 6-8 mm punch on the area previously circled with a permanent marker during SE examination and corresponding to E2 area, was immediately placed in neutral-buffered 10% formalin, trimmed, routinely processed, paraffin embedded and stained with haematoxylin and eosin for histological examination. Histopathological images were observed under an Olympus BX51 photomicroscope equipped with an Olympus C-5060 Wide Zoom and DP software digital camera (Olympus, Tokyo, Japan) for computer assisted image acquisition and analysis.
The cytological and histopathological results were correlated with standard ultrasound and elastographic evaluations.

Statistical analysis
Statistical analysis was performed using JMP® software (version 13; Sas Institute Inc., Cary, North Carolina, USA).
B-mode and elasticity parameters (E-score, E-index, E2 and SR) were correlated with the continuous variables age, sex, body weight and body condition score.
The relationship between cytology, echogenicity, eco-structure, calcification, margin, vascularization and histopathological results was analyzed via Pearson's chi-square test. Elasticity parameters were analyzed using Anova test followed by the post hoc Tukey HSD test. Mann–Whitney test followed by Bonferroni correction were used for the analysis of normally and not normally distributed variables, respectively in order to identify a significant difference between histopathological results.
A significance level of $P < 0.05$ was defined for all tests.
The 75th percentile of the distribution of data was determined in order to calculate a significant optimal cut-off points for differentiating among three histopathological types of diagnosis. Contingency tables were used to summarize the relationship between several groups of variables.
Pearson's chi-square test evaluated the relationship between categorical variables; moreover, R^2 and 95% confidence intervals (CIs) were estimated. The mean values

and standard error (SEM) of elasticity parameters were calculated according to the histopathological results, respectively. Differences between the mean values for the two independent benign and malignant groups were compared statistically by using the student's t-test as alpha=0.017 as significant level.

Results

Study population

Fifty-one dogs met the inclusion criteria: 24 dogs were males (5 neutered) while 27 were females (23 neutered). The mean age was 9±2 years (range=3-13 years). The mean body weight was 27.4±12.85 (range=6-60 kg) and the mean body condition score was 3.41±0.6 (range=2-5). The most highly represented breed was Mixed Breed (n=9) followed by Labrador Retriever (n=5), Boxer (n=4), English Cocker Spaniel (n=2), English Setter (n=2), Dobermann (n=2), Golden Retriever (n=2), French Bulldog (n=2); Pitbull (n=2), Dogo Argentino (n=2), German Shepherd (n=1), Hovawart (n=1), Collie (n=1), English Bulldog (n=1), Jack Russel Terrier (n=1), Dachshund (n=1), Miniature Schnauzer (n=1), Welsh Terrier (n=1), Doberman Pincher (n=1), Italian Spinone (n=1), Newfoundland (n=1), Deutsch Kurzhaar (n=1), Dogue de Bordeaux (n=1), Rhodesian Ridgeback (n=1), Yorkshire Terrier (n=1), West Highland White Terrier (n=1), Bernese Mountain Dog (n=1), Presa Canario (n=1), Italian Bracco (n=1).

No significant association was found between the continuous variables age, body condition score and body weight with standard ultrasound and elastographic evaluations. No significant difference was detected between males and females. Overall, sixty-five skin nodules were clinically evaluated using inspection and palpation and their longest diameter was measured before ultrasound examination.

Standard ultrasound evaluations

Standard ultrasound assessments (size, echogenicity, echo-structure, calcification, vascularization, margins) were evaluated and recorded in all cases.

Mean value of the longest diameters estimated on the B-mode image was 1.9±0.97 cm: the biggest measured 3.85 cm while the smallest 0.5 cm.

Statistically significant relationship between echogenicity, echo-structure, calcifications, vascularization, size and the final histopathological diagnosis were not identified (P>0.05).

Standard ultrasound reported fifty-three hypoechoic (81.53%), nine isoechoic (13.8%) and three hyperechoic nodules (4.6%). Inflammatory nodules tended to appear hypoechoic (n=15; 88.2%); benign nodules were classified in 25 hypoechoic (80.6%) and 6 isoechoic (19.4%). Most of the malignant nodules were hypoechoic (n=13;

243   76.4%); however, 3 also appeared isoechoic and only one hyperechoic. Benign and
244   malignant nodules showed a similar incidence of iso-echogenicity (19.3% and 17.6%,
245   respectively).
246   The 80% (52/65) of skin nodules, diagnosed as inflammatory lesions (n=15; 88.2%),
247   benign (n=23; 65.7%) and malignant tumors (n=14; 82.3%), appeared all
248   inhomogeneous. Three benign tumors (5%) showed calcifications within the
249   parenchyma.
250   Intralesional power and color Doppler was positive in 25 nodules (38.4%), 10 were
251   benign tumors followed by 9 malignant and 6 inflammatory.
252   Finally, a significant relationship was demonstrated between margins and
253   histopathological findings: 26 benign nodules (83%) evidenced the highest incidence
254   of defined margins (R^2=0.14; P=0.004).
255
256   **Strain elastography evaluations**
257   E-index and SR values were normally distributed (mean±SEM 3.57±0.15 and
258   2.27±0.25 respectively). E2 and E-score values were not normally distributed with a
259   median of 3.65 and 2.65, and interquartile range (IQR) of 3 for both. A significant
260   negative correlation was observed between the longitudinal diameter of skin nodules
261   and E-score with a P=0.0002 and r=-0.45 (LCI95% -0.63; UCI95% -0.23) , E-index
262   with a P=0.007 and r=-0.41 (LCI95%-0.6; UCI95%-0.19)and E2 with a P=0.0015 and
263   r=-0.40 (LCI95%-0.6; UCI95%-0.16) respectively.
264   SE features (E-score, E-index, E2 and SR) did not significantly differ between the
265   three histopathological diagnoses (P>0.05).
266   The distributions of E-score values are summarized in Table 2. Malignancies did not
267   show significantly higher elasticity scores (mean±SEM=3.75±0.34) compared with
268   inflammatory (mean±SEM=3.640±0.36) and benign nodules
269   (mean±SEM=3.230±0.24).
270   The distributions of E-index values are summarized in Table 3. A relationship
271   between inflammatory (mean±SEM=3.5±0.3), benign (mean±SEM=3.6±0.23),
272   malignant nodules (mean±SEM=3.56±0.28) and E-index values was not found. On
273   the other hand, a significant association between E-index values and calcification
274   (R^2=0.20; P=0.009) was identified. Calcific nodules showed a significant higher E-
275   index values (mean±SEM=5.43±0.69; CI95%=4.01±6.85) than not calcific (mean±
276   SEM=3.41±0.22; CI95%=2.95±3.87).
277   In addition, a relationship between E-index values and the presence or absence of
278   vascularization was demonstrated (R^2=0.24; P=0.004) because nodules showing a
279   positive Power and color Doppler revealed a higher E-index (mean±SEM=4.05±0.25;
280   CI95%=3.53±4.58) than negative (mean±SEM=2.6±0.37; CI95%=1.91±3.42).
281   The distributions of E2 values are summarized in Table 4. Malignancies did not show
282   significantly higher elasticity scores (mean±SEM=3.6±0.32) compared with
283   inflammatory (mean±SEM=3.67±0.43) and benign nodules (mean±SEM=3.56±0.30).

284   The distributions of SR are summarized in Table 5. SR values did not significantly
285   differ between inflammatory (mean±SEM=1.94±0.45), benign
286   (mean±SEM=2.27±0.29) and malignant nodules (mean±SEM=2.03±0.40).
287   An overall difference between groups after elimination of the outlier value 10,7 was
288   not confirm and, when we considered a value of 3,325 corresponding to 75
289   percentiles as the optimal SR cut-off for the purpose of determining malignancies, an
290   overlap in this parameter was demonstrated between benign and malignant nodules.
291   In addition, nodules rich in keratin debris in cytological evaluation revealed the
292   highest SR (mean±SEM=3.68±0.49; CI95%=2.67±4.7) among the cytological
293   diagnoses (R^2=0.35; P=0.008).
294   Finally, mast cell tumors showed the highest E-score (mean±SEM=4±0.37;
295   CI95%=3.24-4.75) and follicular benign tumors showed the highest SR
296   (mean±SEM=2.56±0.38; CI95%=1.77-3.34) between cancer groups, but statistically
297   significant difference was not detected.
298

299   **Fine-needle cytological results**
300   Cytologic findings were inconclusive in 19 cases (29.2%) due to poor cellularity of the
301   cytologic specimen. Fine-needle cytology was diagnostic for 42 skin nodules (64.6%):
302   12 were inflammatory lesions, 17 benign and 13 malignant tumors. A significant
303   association was identified between cytological and histopathological results (R^2=0.29;
304   P< 0.001). Cytology showed a sensitivity of 75% and specificity of 100% for
305   diagnosing malignant neoplasia.
306

307   **Histopathological results**
308   Of the sixty-five nodules examined, 47.6% (n=31) were benign followed by malignant
309   (n=17; 26.15%) and inflammatory nodules (n=17; 26.15%). Among the inflammatory
310   nodules, deep pyoderma was frequent (n=5, 29.4%). The most common benign
311   neoplastic lesion was hair follicular tumor (n=13; 41.9%), followed by lipomas (n=7;
312   22.58%). Mast cell tumor was the most represented malignant tumor (n=10; 58.8%).
313   The histopathologic diagnoses are listed in Table1.
314

315   **Discussion**
316   In this study, SE combined with standard ultrasound was used to differentiate
317   inflammatory from benign and/or malignant neoplastic skin nodules in dogs. Both
318   qualitative and semiquantitative evaluations were performed and novel and more
319   accurate elasticity parameters as E2 and SR were also analyzed for the first time.
320   Unfortunately, except for the highest E-score and SR values observed for mast cell
321   and hair follicular tumors between cancer groups and cases in which SE was not
322   recommended because produced falsely stiff images, no other satisfactory results
323   and pathognomonic SE patterns were demonstrated to better predict malignancy.
324   In humans, most of the early work on the use of acoustic methods for the
325   assessment of elastic properties of soft tissues is focused on skin because many

326   dermatologic diseases manifest through changes in cutaneous mechanical
327   properties, and skin is the most accessible soft tissue.[23-25] On the contrary, there has
328   been limited research in veterinary dermatology. For example, only in a previous
329   study, SE was demonstrated as a novel, noninvasive, and complementary tool for
330   differentiating malignant from benign lipomatous skin lesions in dogs.[21] However,
331   some consideration should be considered when comparing these results. Since SE
332   involves two common elements that are the application of a force or stress to
333   calculate local strain, and the measurement of a mechanical response, the main
334   limitation is that we have no knowledge of the stress we really apply. Indeed, the
335   method is freehand and it is difficult to perform numerical evaluations of the tissue
336   elastic properties to objectively make comparisons between cases.[1-7] Moreover, for
337   many applications where the distinction between a benign and malignant lesion is
338   crucial, consecutive series have shown that the specificity of this method is
339   satisfactory especially when it is used in a combined modality.[9-11;26-28]
340   Based on this background, SE in addition to B-mode and US with Doppler were used
341   in this study. Moreover, in order to provide further information on the elasticity of skin
342   nodules, several qualitative and semi-quantitative SE parameters were also
343   evaluated.
344   Elasticity score scales are qualitative and operator-dependent systems that have
345   been used in a wide spectrum of human diseases for detecting elastography patterns
346   and classifying them in the range of benign or malignant lesions.[5,6] Currently, peculiar
347   five-point subjective scoring systems, based on the degree and distribution of strain
348   on SE image, are widely used to screen for breast, lymph nodes, thyroid and prostate
349   cancer.[29-32] Although variations were observed, lesions with a higher score had a
350   higher probability of malignancy.
351   In veterinary medicine, the qualitative elastic scales normally used in humans have
352   been modified in several studies.[33,34] For example, the Tsukuba scale usually
353   adopted in humans to differentiate benign from malignant breast and thyroid
354   nodules,[29,35] has been used in dogs to identify differences between lipomas and other
355   subcutaneous neoplasms, with a 100% specificity and 61% sensitivity.[21]
356   In this study, the Alam five-point scale was used to assess the E-score values. The
357   selection of this scoring system that has been exclusively created for lymph nodes,[22]
358   was ideally supported by the fact that these organs are more similar to skin nodules
359   as far as shape and peripheral localization. However, no statistical correlation was
360   demonstrated between histopathological and E-score values. This result is not
361   surprising if we consider that the subjective evaluation is the most striking feature of
362   elastic scoring system detection and could produce inaccurate classification of skin
363   nodules.
364   Thus, in order to provide more objective qualitative strain data, the color-coded
365   images were analyzed by the software and E-index was reported as the numerical
366   expression of the relative strain value calculated in a user-selected circular area.

367 Surprisingly, the maximum values that correlated with the increased stiffness were
368 assigned to benign lesions.
369 Once again, our results differed from those detected in the previous study that
370 documented the usefulness of hardness cutoff of 50.25% of intralesional hard areas
371 to predict malignancies with a 100% specificity and 89% sensitivity.[21] Therefore, our
372 hypothesis is that differences in performance across these studies were attributable
373 to the inclusion criteria, calibration of the equipment and divergences from the gold
374 standard (cytology or histology) for comparative statistical analysis.
375 On the other hand, when the group of malignant nodules was analyzed, mast cell
376 tumors evidenced an elevated incidence of higher E-score and E-index values, but a
377 statistically significant difference was not detected. This variability may be the result
378 of cell density or arrangement, but further studies are necessary to better understand
379 this finding. Of note, however, statistical results revealed that SE is not the best
380 method to screen some cutaneous nodular lesions because of possible false-
381 negative results.
382 For example, the statistically higher E-index values were detected in the calcified
383 nodules. This finding corresponded to what reported for calcifications in human
384 thyroid nodules that may produce misleading elastic measurements and the increase
385 of their stiffness.[36]
386 Interestingly, in this study a significantly negative correlation between the parameters
387 size and elasticity was detected and we emphasized that size of lesion influenced the
388 degree of hardness. This data confirms the main limit of SE technique: with the
389 increasing size of the lesions, the surrounding normal soft tissue is not adequately
390 imaged into the ROI box, making its displacement less effective and causing falsely
391 stiff images.[1-4] In humans a similar restriction is well-known: recently, thyroid nodules
392 reported sensitivities of 86% and 65% and specificities of 100% and 62% for lesions
393 <2 cm and >2 cm in diameter, respectively.
394 Finally, in this study vascularized nodules consistently displayed greater E-index
395 values compare to the other groups. This finding is not surprising if we consider that
396 fluids cannot be mechanically compressed and act as stress dampers limiting tissue
397 movement.[1-4] Thus, inflammatory lesions and benign follicular tumors characterized
398 by cystic areas full of tissue debris or with positive Doppler obtained the same
399 outcome.
400 For the first time, in this study another elasticity parameter, the E2 was evaluated to
401 further correlate the results. Unfortunately, E2 did not allow to highlight the utility of
402 SE as an adjunctive technique for cutaneous nodule analysis. However, since the
403 stress is not recorded as it travels from the stress source through the tissue as it
404 gradually attenuates, calculating the Elastic modulus from strain data alone is not
405 useful. It was thus used a highly valuable and more objective parameter called SR
406 that expresses a momentarily and relative difference in compressibility in two user-
407 selected areas within selected regions of interest in a strain elastogram.[37]

408 Some reports in veterinary medicine have evaluated the application of SR. [33,38-40]
409 However, based on our knowledge, no previous study has reported the use of this
410 parameter to discriminate between several skin nodules of different origin. Using
411 machine inherent software, twelve nodules obtained a value < 1 displaying softer
412 than the surrounding healthy tissue. Surprisingly, five of these nodules were
413 malignant. Skin nodules resulted rich in keratin debris in cytological reports showed a
414 significant higher SR when compared with other subtypes probably due to their mixed
415 and cystic composition. Moreover, an optimal SR cutoff helpful to detect malignancy
416 was not revealed because an overlap between these values in both benign and
417 malignant nodules was found.
418 Differences among our results and those of some human studies that reported how
419 SE plus SR improved significantly the accuracy of SE for discriminating skin tumors
420 can be partly explained by several observations. For example, diversity between the
421 position of the ROI in healthy and pathological tissue is a possible variable that
422 should be contemplated. Although better results for measurements of SR are taken
423 at the same depth of the target nodules, it is not always possible to respect what is
424 recommended by the manufacturers in clinical practice. Moreover, the variation in the
425 elasticity of pathological tissues complicates the reproducibility of SR. Skin nodules
426 could have contained ducts and veins that acted as stress dampers, as well as
427 connective tissue induced by malignant cells (desmoplasia) that promote the
428 surrounding tissue induration, limiting its movement. Furthermore, another possibility
429 is that an extension of cancerous tissue peripherally to the nodule was not interpreted
430 adequately. If this occurred, tissue selected as healthy tissue could also contain
431 neoplastic cells. Finally, in five nodules it was impossible to perform SR due to the
432 insufficient amount of perilesional tissue to be included within the elastogram. Based
433 on these findings, strain-imaging artefacts may reduce the feasibility of SR
434 measurements and make this parameter impractical to compute in poorly defined and
435 very superficial skin nodules.
436 Pathognomonic SE patterns useful to better identified skin nodules were not
437 evidenced. The two most commonly described in humans are the 'BGR sign' and
438 'bull's eye artifact that discriminate with good sensitivity solid from cystic lesions.[41,42]
439 We enrolled four benign hair follicle tumors containing cystic areas but in none of
440 them we reported these pathognomonic patterns.
441 Finally, we consider the hypothesis that the differences in body fat could likely
442 influence elasticity assessment of skin nodules, but no relationship was found
443 between the continuous variables body condition score and body weight with
444 elastosonographic assessments.
445 In this study, differences between benignant and malignant nodules were noted
446 depending on the standard ultrasound parameter accounted for.
447 Statistical analyses did not identify any significant relationships between
448 echogenicity, echo-structure, calcification, vascularization and the histopathological
449 diagnosis. Regarding echogenicity, inflammatory and malignant lesions tended to

appear hypoechoic. Benign tumors were more isoechoic, probably because of high incidence of lipomas in this group. The heterogeneous aspect of skin nodules was common and most likely related to vascularization and fibrous stroma often described in histopathological reports. Benign and malignant tumors were inclined to display a positive intralesional vascularization, but a statistically significant difference was not demonstrated.

Of note, however, margin regularity was useful for distinguishing among different nodules. Benign tumors showed the highest statistically incidence of defined margins most likely because they were less infiltrative than the others.

These results were in good agreement with those obtained by previous reports and confirmed that B-mode ultrasound technique alone is less sensitive in detecting malignancies.[43-45]

Follicular tumor, mast cell tumor and lipoma were the most common histopathological diagnosis and association between fine-needle cytological and histopathological results was obtained.

In conclusion, the current study has some limitations. SE is a free-handed compression type elastography. Although validity and reliability to evaluate several cutaneous diseases were demonstrated, SE is an operator-dependent technique and is based on qualitative without quantitative elasticity evaluations. Distorted signals produced by the excessive or not uniform probe compressions on the skin nodule could produce nonlinearity waves resulting in a false stress concentration, condition which influences the elastic features of SE image. Secondly, the resolution of 10 MHz frequency probe used could be resulted inadequate during scanning of thin lesions such as cutaneous angiosarcoma and squamous cell carcinoma. Currently, high-frequency ultrasound transducers (20-100 Mhz) are frequently used in humans but are uncommon available in veterinary medicine. Furthermore, skin is more vulnerable to possible artifacts than other organs because oscillations deform superficial tissues closer to the ultrasound transducer more than deep parenchymas. In addition, the inclusion of skin nodules located on sharply curved parts of the body or closed in proximity of the underlying bone could have negatively influenced our results. The instable positioning of the transducer generates a poor contact skin-probe and a not-uniform compressions. Consequentially, distorted images and falsely elastosonographic estimations can have been produced.

A further limitation was that only one most representative image, corresponding to the best approximation of the slice that would be interpreted on histopathology, was used to assess elastosonographic evaluations. This choice could have influenced the ability to capture the totality of elasticity information for each skin nodule.

Finally, the lack of statistical difference for elasticity parameters as well as the absence of a pathognomonic pattern helpful to characterize skin nodules could have been influenced by the small group of dogs enrolled and the reduced variety of histopathological subtypes.

In summary, the results of this study suggest that although SE is a feasible and non-invasive technique, an overall diagnostic performance during the routine clinical practice for discriminating skin nodules affected by different diseases was not found. Malignancies were not characterized by more changes in tissue stiffness and the use of classic and novel SE evaluations did not uncover pathognomonic elastic patterns of malignant and benign skin lesions.

Future research will be needed to evaluate the elastic properties of normal skin and subcutaneous tissue in order to improve the diagnostic value of this relatively unexplored frontier in skin imaging for better discriminate among several skin diseases in dogs.

References

1) Dewall RJ. Ultrasound elastography: principles, techniques, and clinical applications. Crit Rev Biomed Eng. 2013; 41:1-19.

2) Gennisson JL, Deffieux T, Fink M et al. Ultrasound elastography: principles and techniques. Diagn Interv Imaging. 2013; 94: 487-495.

3) Sigrist RMS, Liau J, El Kaffas H et al. Ultrasound Elastography: Review of Techniques and Clinical Applications. Theranostics. 2017; 7:1303–1329.

4) Ophir J, C´espedes I, Ponnekanti H et al. Elastography: a quantitative method for imaging the elasticity of biological tissues. Ultrason Imaging 1991; 13:111–134.

5) Shiina T, Nightingale KR., Palmeri ML et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: basic principles and terminology. Ultrasound in medicine & biology 2015; 41: 1126-1147.

6) Ozturk A, Grajo JR, Dhyani M et al. Principles of Ultrasound Elastography. Abdom Radiol 2018; 43: 773–785.

7) Bamber J, Cosgrove D, Dietrich CF et al. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1. Basic principles and technology. Ultraschall Med2013; 34:169–184.

8) Dietrich CF, Barr RG, Farrokh A et al. Strain Elastography – How To Do It? Ultrasound Int Open. 2017;3: 137–149.

9) Dasgeb B, Morris MA, Mehregan D et al. Quantified ultrasound elastography in the assessment of cutaneous carcinoma. Br J Radiol. 2015; 88: 20150344.

526 10) Botar-Jid CM, Cosgarea R, Bolboaca SD et al. Assessment of cutaneous
527 melanoma by use of very- high-frequency ultrasound and real-time elastography. Am
528 J Roentgenol 2016; 206: 699–704.

529 11) Park HJ, Lee SY, Lee SM et al. Strain elastography features of epidermoid
530 tumors in superficial soft tissue: differences from other benign soft-tissue tumors and
531 malignant tumors. Br J Radiol 2015; 88: 20140797.

532 12) DeJong HM, Abbott S, Zelesco M et al. The validity and reliability of using
533 ultrasound elastography to measure cutaneous stiffness, a systematic review. Int J
534 Burns Trauma 2017; 7: 124–141.

535 13) Iagnocco A, Kaloudi O, Perella Cm et al. Ultrasound elastography assessment of
536 skin involvement in systemic sclerosis: lights and shadows. J Rheumatol. 2010; 37:
537 1688-1691.

538 14) Gaspari R, Blehar D, Mendoza M et al. Use of ultrasound elastography for skin
539 and subcutaneous abscesses. J Ultrasound Med 2009; 28: 855–860.

540 15) Nesje L, Havre R, Waage J et al. Strain ratio as a quantification tool in strain
541 imaging. Appl Sci 2018; 8: 1273.

542 16)Holdsworth A, Bradley K, Birch A et al. Elastography of the normal canine liver,
543 spleen and kidneys. Vet Radiol Ultrasound 2014; 55: 620-627.

544 17)White J, Gay J, Farnsworth R et al. Ultrasound elastography of the liver, spleen
545 and kidneys in clinically normal cats. Vet Radiol Ultrasound 2014; 55: 428-434.

546 18)Feliciano MAR, Maronezi MC, Pavan L et al. ARFI elastography as a
547 complementary diagnostic method for mammary neoplasia in female dogs:
548 preliminary results. J Small Anim Pract 2014; 55: 504-508.

549 19)Alder D, Bass D, Spörri M et al.Does real-time elastography aid in differentiating
550 canine splenic nodules? Schweiz Arch Tierheilk 2013; 155: 491-496.

551 20) Mantziaras G, Luvoni GC. Ultrasound elastography of the normal canine prostate
552 and testicles. International Symposium on Canine and Feline Reproduction, 2016.
553 (Short communication)

554 21)Longo M, Bavcar S, Handel I et al.Real-time elastosonography of lipomatous vs.
555 malignant subcutaneous neoplasms in dogs: Preliminary results. Vet Radiol
556 Ultrasound 2017; 59: 198-202.

557 22) Alam F, Naito K, Horiguchi J et al. Accuracy of Sonographic Elastography in the
558 Differential Diagnosis of Enlarged Cervical Lymph Nodes: Comparison with
559 Conventional B-Mode Sonography. Am J Roentgenol 2008; 191: 604-610.

560 23) Pierard GE, Lapière CM. Physiopathological variations in the mechanical
561 properties of skin. Arch Dermatol Res 1977; 260: 231-239.

562 24) Agache PG, Monneur C, Leveque JL et al. Mechanical properties and Young's
563 modulus of human skin in vivo. Arch Dermatol Res 1980; 269: 221-232.

564 25) Pereira JM, Mansour JM, Davis BR. Analysis of shear wave propagation in skin;
565 application to an experimental procedure. J Biomech 1990; 23: 745-751.

566 26) Wojcinski S, Farrokh A, Weber S et al. Multicenter study of ultrasound real-time
567 tissue elastography in 779 cases for the assessment of breast lesions: improved
568 diagnostic performance by combining the BI-RADS(R)-US classification system with
569 sonoelastography. Ultraschall in Med 2010; 31: 484-49.

570 27) Brock M, Von Bodman C, Palisaar RJ et al. The impact of real-time elastography
571 guiding a systematic prostate biopsy to improve cancer detection rate: a prospective
572 study of 353 patients. J Urol 2012; 187: 2039-2043.

573 28) Trimboli P, Guglielmi R, Monti S et al. Ultrasound sensitivity for thyroid
574 malignancy is increased by real-time elastography: a prospective multicenter study. J
575 Clin Endocrinol Metab 2012; 97: 4524–30.

576 29) Itoh A, Ueno E, Tohno E et al. Breast disease: clinical application of US
577 elastography for diagnosis. Radiology 2006; 239: 341-350.

578 30) Zhao CK, Xu HX. Ultrasound elastography of the thyroid: principles and current
579 status. Ultrasonography 2019; 38:106–124.

580 31) Dudea SM, Botar-Jid C, Dumitriu D et al. Differentiating benign from malignant
581 superficial lymph nodes with sonoelastography. Med Ultrason 2013; 15:132-139.

582 32) Correas JM, Tissier AM, Khairoune A et al. Ultrasound elastography of the
583 prostate: state of the art. Diagn Interv Imaging 2013; 94: 551-560.

584 33) Barella G, Lodi M, Faverzani S. The role of strain elastography in the evaluation
585 of canine hypoechoic splenic lesions. In: BSAVA. British small animal veterinary
586 association, 2015; 466-466.

587 34) Seiler GS, Griffith E. Comparisons between elastographic stiffness scores for
588 benign versus malignant lymph nodes in dogs and cats. Vet Radiol Ultrasound 2018;
589 59: 79-88.

590 35) Rago T, Vitti P. Role of thyroid ultrasound in the diagnostic evaluation of thyroid
591 nodules. Best Pract Res Clin Endocrinol Metab 2008; 22: 913–928.

592 36) Rago T, Santini F, Scutari M et al. Elastography: new developments in ultrasound
593 for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab 2007; 92:
594 2917–2922.

595 37) Havre RF, Waage JER, Mulabecirovic A et al. Strain Ratio as a Quantification
596 Tool in Strain Imaging. Appl Sci 2018; 1273

597 38) Jeon S, Lee G., Lee S et al. Ultrasonographic elastography of the liver, spleen,
598 kidneys, and prostate in clinically normal beagle dogs. Vet Radiol Ultrasound 2015;
599 56: 425-431.

600 39) Di Francesco P, Petrizzi L., Guerri G et al. Ultrasound elastography for evaluation
601 of tendons and joint capsules in healthy dogs and horses: a preliminary study. J Med
602 Ultrasound 2012; 20: 79-86.

603 40) Lee G, Jeon S, Lee S et al. Strain elastography using dobutamine-induced
604 carotid artery pulsation in canine thyroid gland. Vet Radiol Ultrasound 2015; 56: 549-
605 553.

606 41) Cho N, Moon WK, Chang JM et al. Aliasing artifact depicted on ultrasound (US)-
607 elastography for breast cystic lesions mimicking solid masses. Acta Radiol 2011; 52:
608 3–7.

609 42) Barr RG, Lackey AE. The utility of the "bull's-eye" artifact on breast elasticity
610 imaging in reducing breast lesion biopsy rate. Ultrasound Q. 2011; 27: 151–155.

611 43) Volta A, Bonazzi M, Gnudi G et al. Ultrasonographic features of canine lipomas.
612 Vet Radiol Ultrasoun 2006; 47: 589-591.

613 44) Park JK, Hong IH, Ki MR et al. Multiple perianal infundibular follicular cysts in a
614 dog. Vet Derm 2010; 21: 303-306.

615 45) Nyman HT, Kristensen AT, Lee MH et al. Characterization of canine superficial
616 tumors using gray-scale b mode, color flow mapping, and spectral doppler
617 ultrasonography—a multivariate study. Vet Rad Ultrasound 2006; 47: 192–198.

618