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Feasibility of shear wave elastography (2D -SWE) to evaluate cristalline lens in healthy dogs

Giovanni Aste[1], Massimo Vignoli[1], Sonia Panzeri[1], Roberto Tamburro[1], Francesco Simeoni[1], Andrea De Bonis[1], Martina Rosto[1] and Francesca Del Signore[1*]

Abstract

Background 2D shear wave elastography (2D SWE) is an emerging technique in veterinary medicine able to assess tissue stiffness in a non-invasive way. Nowadays no report is yet available about its application in assessing the mechanical properties of canine lenses.

Objectives This study aimed to evaluate the repeatability and reproducibility of 2D SWE in assessing normal lens elasticity in healthy and ageing dogs.

Methods Trans-corneal lens 2D SWE was performed under physical restraint on 33 dogs by two operators who collected triplicate kPa and m/s measures, with the aim to assess reproducibility and reliability of the technique, followed by the evaluation of eventual difference of stiffness in different ages (G1 < 1.5 years, G2 1.5 years-7 years and G3 > 7 years). The project has been approved by the CEISA Ethical Committee (Prot. N. 12/2019 361 CEISA). Written informed consent was obtained by all the owners.

Results Mean elasticity values were respectively 4.78 ± 1.48 m/s and 74.9 ± 43.7 kPa for the left eye and 4.45 ± 0.98 m/s and 75.9 ± 43.6 kPa for the right eye. Despite a slight difference observed in the measurements obtained in m/s between the two operators, the intra-observer assessment was excellent in the overall population of dogs for both values in KPa and m/s, as well as the inter-observer one (ICC > 0.75). All the sCV% computed evidence a low measurement dispersion (< 12%). Mean lens stiffness for G1 was 3.1 ± 0.5 m/s and 28.9 ± 9.3 kPa, for G2 4.61 ± 0.62 m/s and 65 ± 18.4 kPa and for G3 6.46 ± 0.36 m/s and 126 ± 14.5 kPa; a significant difference P (< 0.001) was detected between all the groups.

Conclusions It can be concluded that 2D SWE is a rapid and non-invasive US-based technique able to assess lens mechanical properties in companion animals since it is characterized by high reliability and reproducibility, providing also information regarding lens stiffness in aging dogs.

Keywords Lens, Dog, US, 2D shear wave elastography

*Correspondence:
Francesca Del Signore
fdelsignore@unite.it
[1]Department of Veterinary Medicine, University of Teramo, Località Piano D'Accio, Teramo, Italy

Background

The term ultrasound elastography (USE) is referred to a group of Ultrasound (US) based techniques able to assess tissue elasticity in a non-invasive way [1]. This method relies on the basic principle that each tissue is characterized by a certain elasticity, that may be altered in case of specific physiologic and pathologic conditions [2]; in particular, many solid tumors are known to differ mechanically from surrounding healthy tissues and fibrosis associated with chronic diseases causes the tissue to become stiffer than normal tissues [1].

In recent years several USE methods have been developed, allowing the evaluation of tissue elasticity in both qualitative and quantitative ways; the main common physical principle is that the application of mechanical stress to a target tissue will deform the target tissue proportionally to its intrinsic elasticity [1].

Elastography assesses tissue elasticity, which is the tendency of tissue to resist deformation with an applied force, or to resume its original shape after removal of the force [1].

Based on the specific technical settings, US elastography includes qualitative, semiquantitative quantitative techniques, based on the evaluation of tissue elasticity; the most used techniques include strain elastography (SE), acoustic radiation force impulse (ARFI), and 2D shear-wave elastography (SWE) [3].

The SE technique is based on the principle that the tissue elasticity can be measured under a strain that can be manually generated by external compression using an ultrasound probe.

The acquired tissue elasticity can be qualitatively evaluated assessing the color distribution obtained by the software provided analysis; data, indeed, is encoded as color-mapping (typically red is for soft tissues and blue is for hard tissues) over the B-mode images.

Some of the available softwares allows a semiquantitative tissue evaluation; a pseudo-quantitative measurement called the strain ratio can be used, consisting in the ratio of strain measured in adjacent (usually normal) reference tissue region of interest (ROI) to strain measured in a target lesion ROI. A strain ratio < 1 indicates that the target lesion compresses more than the normal reference tissue, indicating higher strain and lower stiffness [4].

The ARFI elastography technique is able to provide quantitative and qualitative measurements of tissue stiffness based on short, high-intensity acoustic pulses to deform the tissue and create a static greyscale map (elastogram) that represents the relative stiffness of the tissue in the imaged region.

In brief, focused, short-duration acoustic push pulses travelling along the main US beam induce within tissues shear stresses, with modalities and intensities depending upon tissue attenuation based on their intrinsic elasticity; the final output of the software analysis is an "elastogram", a visual representation of tissue elasticity.

The elastogram can be then qualitatively compared to the corresponding conventional ultrasound image evaluating the color distribution, in general, lighter areas represent more elastic tissue compared with darker areas; alternatively, the quantitative approach to ARFI utilizes a primary acoustic impulse sent towards a region of interest and promotes the formation of pressure waves, that can be measured and express the tissue elasticity based on the tissue speed propagation expressed in m/s [1, 4].

2D-SWE is another quantitative technique that is able to induce shear waves in multifocal zones and measures the shear waves (SWS) that propagates at several locations around the acoustic pulse through a detection pulse, through a "continue" tissue interrogation" with simultaneous anatomical B-mode US imaging.

Subsequently, SWS inside the field of view (FOV) is integrated and the distribution of SWS is displayed through a color map showing different grades of stiffness, with blue and red colors expressing the highest or the lowest elasticity based on the software technical setting; the average SWS is also quantified as Young's modulus in kilopascals (kPa) and shear wave velocity (SWV) measured in m/s by placing the region of interest (ROI) within the field of view (FOV) [5].

The advantage of this technique compared to ARFI is that real-time visualization of a color quantitative elastogram superimposed on a B-mode image enables the operator to be guided by both anatomical and tissue stiffness information [1, 5], allowing more accurate measurements,

In veterinary medicine, the interest in possible clinical applications of these techniques is rich with several recent reports available regarding the canine liver, spleen, kidneys, musculoskeletal system, lymph nodes, and prostate gland [6–12].

Indeed, since these are US-based techniques and potentially highly operator-dependent, some of these works were also focused on the reproducibility of the technique [9, 11, 13], concluding that the results obtained are reproducible and appliable in routine practice.

In canine species, healthy lenses are basically composed of aqueous content and 33% of protein fibers organized in the lamellae [14].

However, over the course of a normal canine lifespan, increasing numbers of lens fibers occupy the relatively finite space within the lens capsule, subsequently compressing the lens nucleus and increasing its density, thus leading to a physiological phenomenon called nuclear sclerosis (NS) [14].

This is different from cataract, during which, there is a disorganization of the lamellar arrangement and dehydration of the lens, leading to opacity, which results

Table 1 Intraoperator repeatability for measurements expressed in M/s. The significant difference between the sCV% evidenced that O2 collected more repeatable measurements than O1

	CV%	sCV%	ICC
O1	1.64 ± 1.66	4.1 ± 4.13	0.996
O2	1.6 ± 1.17	3.83 ± 3.7	0.995
P		< 0.001	

Table 2 Intraoperator repeatability for measurements expressed in kPa. The significant difference between the sCV% evidenced that O2 collected more repeatable measurements than O1

	CV%	sCV%	ICC
O1	3.40 ± 3.54	6.00 ± 6.25	0.995
O2	2.32 ± 2.34	5.42 ± 5.5	0.998
P		< 0.001	

Table 3 Interoperator reproducibility results

	m/s	kPa
CV%	3.46 ± 3.98	7.7 ± 2.01
sCV%	6.11 ± 7.04	5.85 ± 6.64
ICC	0.994	0.994

Table 4 Age related lens stiffness. Notice the progressive increase of stiffness between the groups

Lens elasticity	Group 1	Group 2	Group 3	*P*
m/s	3.1 ± 0.5	4.61 ± 0.62	6.46 ± 0.36	< 0.001
kPa	28.9 ± 9.3	65 ± 18.4	126 ± 14.5	< 0.001

in variable visual deficiency, according to the stages of development of the condition, thus leading to a change of natural level of stiffness [17a].

While in human medicine the quantification of the visual impairment is easily achieved in the clinical and instrumental eye examination, the same is non currently possible for veterinary patients, who are not able to provide verbal feedback about viewing an image or describe it [14].

Nowadays, the researchers are focusing on provide complementary examinations able to quantify the vision deficits, as for example the refraction; a recent report found that myopia did not increase significantly with increasing NS grade in dogs, although dogs with higher grades of NS had poorer owner-reported vision and were more likely to exhibit higher of myopia than dogs with minimal NS [14].

However, a quantitative and objective system to quantify lens stiffness is not yet available in the clinical practice.

For that reason, ARFI elastography has been investigated as a possible diagnostic technique to be used in case of cataract, and authors assessed that this specific elastography technique is can assess lens elasticity in case of different stages of cataract [15]a.

Nowadays a similar report regarding the feasibility of 2D SWE is not available; for such purpose, authors provided a research work aimed to prove repeatability and reproducibility of 2D Shear Wave elastography on healthy ageing dogs with dual purposes, the first assess the feasibility of the technique as reproducible tool and then test its efficacy to discriminate different levels of stiffness in ageing dogs.

Results

Repeatability and reproducibility

The mean eye and lens size were respectively 1.84 cm±0.15 cm and 0.64 cm±0.04 cm for both the left and right eye, with no significant difference between the left and right side ($p = 0.75$ and 0.79 respectively).

The overall mean stiffness recorded by both operators on both the left and right sides was 4.78±1.48 m/s for O1 and 4.45±0.98 m/s for O2 and 74.9±43.7 kPa for O1 and 75.9±43.6 kPa for O2; a slightly significant difference ($p = 0.03$) was observed between the two operators for m/s measurements, while no significant difference was detected for kPa measurement ($p = 0.06$).

In Table 1 intra-observer CV% values for each operator are summarized; the significant difference of sCV% between the two operators points that that O2 collected more repeatable measurements than O1 (Tables 1 and 2).

As far as it regards the intraobserver repeatability for the measurements, the ICC was classified as excellent for both m/s and kPa (Tables 1 and 2).

Interobserver CV% was very low, with no significant difference of inter-observer sCV% for kPa and m/s in overall measures ($P = 0.06$) detected between the two operators.

The interobserver reproducibility was classified as excellent too, with an ICC of 0.94 (Table 3).

Age-related lens stiffness

In Table 4, mean values for m/s and kPa of each group are resumed. A progressive increase of stiffness was recorded in aging dogs, with a significant difference ($P < 0.001$) observed between all the groups nor between the mean value from O1 and O2.

Discussion

In this work, 2D SWE was investigated as a possible US-based method to assess lens elastic properties in healthy dogs; the choice to perform such investigation relies on the current increasing interest for elastography-based techniques to assess and quantify elastic tissue properties of ocular structures, and in particular, lens in both human and veterinary medicine.

In human medicine, for example, SE has been investigated as a possible technique to assess the progressive lens nucleus sclerosis in aging patients, and the results obtained proved that the progressive and physiologic increase of lens stiffness can be detected with SE in vivo and it provides a valuable method for clinical grading [16].

SWE has been investigated as a possible technique to quantify lens softening to treat presbyopia on a phantom-based model, with encouraging results to be applied in the clinical practice [17].

In veterinary medicine, in particular for canine species, a recent report described the results obtained with the application of ARFI elastography on canine lenses affected by different types of cataract [15].

The authors observed that this technique is useful to assess the increasing of the lens stiffness in the diseased lens, and the different levels of rigidity based on different underlying disorders; in particular, diabetic cataract provided lower stiffness compared to mature or hypermature cataracts [15].

The current increasing interest for sonoelastography-based technique in ophthalmology led the authors to investigate the feasibility of 2D SWE to quantify lens stiffness in healthy dogs.

This specific software has been chosen by the authors since it requires no manual pressure exercised on the probe, thus making it more tolerable by awake dog; the idea of the authors was, indeed, to perform a protocol less invasive as possible and test it patients without the need of sedation.

Indeed, the basic technique of 2D SWE consists of keeping the probe still on the target surface without manually cause tissue deformation with manual compression, as it happens with other techniques such as Strain Elastography; this reduces the influence of the single operator pressure, but it requires the ability of the clinicians to keep the probe still without pressure, in order to avoid bias of the measurements [2].

The main focus in this phase of the investigation was the assessment of repeatability and reproducibility of the measurements, so to provide a baseline for a large-scale appliable protocol.

Indeed, the quality of an US examination is highly operator-dependent, and this is valid also for sonoelastography-based techniques, whose results can be affected by various factors.

Their effect on reliability and reproducibility of sonoelastography 2D SWE according to the organs applied has been investigated in humans and guidelines for liver, breast, thyroid, and prostate elastography with 2D SWE have been established for proper shear wave induction (2,5,6,20); the guidelines suggest specific recommendations about the patient's factors such as fasting, position, access, and breathing and also the technical factors such as depth, location, and size of ROI and the number of measurements.

The assessment of repeatability and reproducibility has been performed in veterinary medicine too, to assess potential influencing factors that may impair the feasibility of the technique.

Next to the potential role of influencing factors such as anesthesia or depth of the measurements, whose discussion is beyond the scope of the current paper and specific details are described in specific papers (reproducibility of SE and SWE), both SE and 2D SWE were defined repeatable and reproducible techniques regarding the influence of the operator to collect the measurements [7, 9, 13, 19–21].

The results of the current paper evidence that measurements are highly repeatable and reproducible, even if slight differences have been detected.

To standardize the procedure, both operators gently applied the probe on the ocular surface without exercising pressure on the corneal surface; keeping a highly deformable anatomical reference such as the corneal surface as a marker of the pressure exercised was a crucial factor in increasing the reproducibility of the technique.

Indeed, slight differences were observed between operators.

In particular, a significant difference was observed between the measurements collected with m/s but not with kPa, and the difference observed for sCV% between the operators states that O1 was less repeatable than O2.

These differences can be explained considering the free-hand nature of the technique, and even with a clearly standardized protocol, slight differences between operators can be always observed. Since this is a free-hand technique, keeping the exact same pressure by two different operator can be challenging, and even within the same operator; indeed, for this specific software, an auxiliary tool to monitor the manual pressure is not available, as it could be for techniques as strain elastography, where a color-bar active during the examination provides the adequacy of the pressure [22].

The lack of such tool could lead to even slight differences in measurements, however, this difference, even if statistically significant, needs to be contextualized in the clinical practice to address it real influence in the routine practice.

Regarding, in particular, the difference observed in m/s but not in kPa, it must be considered that in anisotropic tissues (as tendons) shear wave velocity may not exactly correspond to the Young Modulus, thus leading to not exact correspondence between m/s and kPa.

This is because to calculate the Young Modulus the tissue density is part of the formula, so in isotropic tissues the correspondence between m/s and kPa is more accurate than in anisotropic tissues [23]; this is the reason why some authors suggest to perform the measurements in m/s for anisotropic tissues, such as tendons [24].

Since in the studies available on dogs and rabbits the measurements were expressed with both m/s and kPa, authors decided to collect measurements with both the units; the results herein described assessed that kPa might be more reproducible than m/s, as it has been observed in a previous report [7].

The application of both two measurements in clinical studies will highlight the real significance of this difference.

This study was conducted to assess a base-line to perform more investigation in diseased dogs; it was crucial, indeed, to provide the reliability of the technique before applying it to investigate lens disorders.

Moreover, the evidence of significant difference in stiffness in ageing dogs, provides that this technique is effective to discriminate the physiological increase of stiffness in ageing lens, as it happens also in human medicine [16, 25].

This could be useful in the clinical evaluation, first to quantify objectively the lens elasticity in and second be associated to other diagnostic technique to quantify eventual loss of vision ability, as it happens in case of myopia [25].

Indeed, the lack of verbal feedback from canine patients limits the clinical information that an ophthalmologist could benefit from to correctly manage the clinical case and, for that purpose, auxiliary tools as 2D SWE could be useful in the routine clinical examination providing quantitative and more objective data.

As far as it regards the presence of cataract, while the diagnosis and clinical monitoring is easily achieved by visual inspection, in case of surgical planning, it should be considered that the success of phacoemulsification surgery depends on the physical properties of the lens, since it has been reported that surgery may fail in case of capsular rexis that may happen in case of an intumescent cataract or case of too hard capsule [17a, 26].

Moreover, in case of medical treatment with drugs such as Lanosterol, in could be associated to the visual inspection to quantify the clinical efficacy of the treatment [27].

This makes 2D SWE a potentially useful technique to assess an excessive lens rigidity and adjust the surgical planning, avoiding to surgically treat subjects at high risk of failure or post-op complications.

However, this technique is not without limitations.

The first limitation of this study relies on the fact that 2D SWE is a technique developed for tissues that should be incompressible, isotropic, and solid, to achieve a reliable Young's Model measurement. In calculating Young's modulus for the lens, it should be considered that the heterogeneous architecture of the eye (i.e. the eye behaves similarly to a poroelastic body, with a solid skeleton incorporating amounts of fluid within its pores) may compromise to some extent, the potential application of the methodology employed in the present study [28].

Therefore, particular attention needs to be paid to visual artifacts that may impair the objective elasticity quantification.

Furthermore, since this technique is highly sensitive to the movement of both operator and patient [29], a standard protocol of chemical constraint could be required to include dogs with different pathological conditions, thus potentially including bias to evaluate tissue stiffness due to change in intraocular pressure or reduced lens visualization in case of eye bulb rotation [28].

Finally, regarding the herein described protocol, the number of collected measurements needs to be discussed.

Current 2D SWE guidelines available for human medicine suggest to collect 5–10 measurements on the target tissue [5, 18]; the authors decided to collect only 3 measurements based on the available literature on animal models with the same software [30], but increase the number of measurements for future clinical studies could improve the diagnostic accuracy of the measurements knowing that the single measurement is repeatable and reproducible.

Conclusion

In conclusion, this study demonstrates the feasibility of 2D SWE as a repeatable and reproducible tool to assess canine lens stiffness properties; this work provides a baseline to perform more studies on clinical applications to assess canine lens stiffness.

Methods

Selection criteria and study population

This is an observational cross-section study that included healthy owned dogs who underwent the 2D-SWE at the Veterinary Teaching Hospital from December 2019 to March 2020.

The project has been approved by the CEISA Ethical Committee (Committee on Animal Research and Ethics of the Universities of Chieti-Pescara, Teramo, 360 L'Aquila and of the Experimental Zooprophylactic Institute of Abruzzo-Molise, Prot. N. 12/2019 361 CEISA).

Written informed owner's consent was obtained in all cases. All dogs were confirmed healthy based on physical examination, CBC, serum biochemistry, a complete ophthalmological examination, and ocular ultrasound.

Calm and compliant dogs were selected to avoid sedation or general anesthesia; no age or breed limitation was considered to include dogs since the aim of this study was to assess the reproducibility of the technique regardless of the specific patient.

Thirty-three dogs between 3 months and 13 years were included in this study. The mean age was 5.65 ± 4.62 years. Twenty-four dogs (*n* = 24) were of mixed breed (medium-large size), Miniature Schnauzer (*n* = 2), Rottweiler (*n* = 1), Labrador Retriever (*n* = 1), Akita-Inu (*n* = 1), Yorkshire Terrier (*n* = 1), Dobermann-Pinscher (*n* = 1), English Setter (*n* = 1), Irish Setter (*n* = 1). The mean weight of all dogs involved was 12.6 ± 9.8 kg.

The dogs were then divided in three groups based on age, respectively group 1 (< 1.5 years) including 10 dogs (*n* = 7 mix breed, *n* = 1 Rottweiler, *n* = 1 Labrador Retriever, *n* = 1 Akita Inu, mean age 6 ± 3.2 months), groups 2 (1.5-7 years) including 12 dogs (*n* = 8 mix breed dogs, *n* = 1 Irish Setter, *n* = 1 Yorkshire Terrier, *n* = 1 Miniature Schnauzer and 1 Dobermann- Pinscher- mean age 4 ± 1.8 years) and group 3 (< 7 years) including 11 dogs (*n* = 9 mix breed dogs, *n* = 1 English setter and *n* = 1 Miniature Schnauzer - mean age 11.3 ± 1.3 years), 5 dogs with NS clinically and sonographically evident with a thin hyperechoic curvilinear line running parallel with the lens capsule evident.

Shear wave elastography (2D-SWE) measurements

The ultrasonography evaluation was performed using a Logiq S8 sonographic system (GE Healthcare, Milan, Italy) with shear wave elastography software and a linear probe of 9 L, 8.5–10 MHz; the frequency used was 10 MHz, to achieve the highest superficial detail possible. The examination was achieved while the dogs were awake. A topical anesthetic (oxibuprocaine chloridrate 4 mg/mL– benoxinate chloridrate eye drops, INTES, Casoria Italy) was applied at a dose of two drops to the cornea one minute prior to the examination [31]. The dogs were positioned in sternal recumbency and physically restrained. Through the application of a water-soluble coupling acoustic gel, the probe was placed on the cornea and oriented to the horizontal plane (transcorneal axial plane) until the distinct visualization of the ocular structures (crystalline lens, anterior camera, and vitreous body) was obtained; care was taken not to exercise pressure on the corneal surface. Measurements of the entire eye and lens were recorded by both operators.

At the end of the examination, the gel was flushed from the eye with a sterile eyewash. In accordance with the results of the studies for the clinical use of SWE in humans [30, 32] the elastographic images of the lens and the ciliary body were longitudinally assessed as specific Regions of Interest (ROI), with standard shape, configuration, and surface area, based on the built-in software of the system (GE Healthcare, Milan, Italy) (Fig. 1).

Once the image quality was adequate, the ROI of 0.6 cm was delineated, and elasticity measurements were taken for each ROI in m/s and kPa (Figs. 2 and 3).

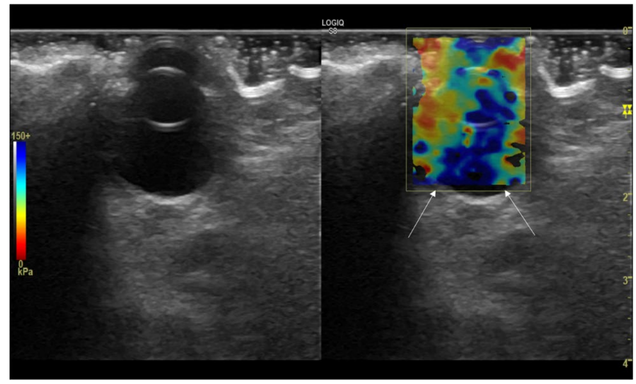

Fig. 1 On the left side of the image, the B-mode image of the eye is presented, with the same B-mode image with the superimposition of the color map on the right ride (white solid arrows). The colorbar present on the left side of the image codifies the tissue stiffness, with blue for hard tissues and red for soft ones

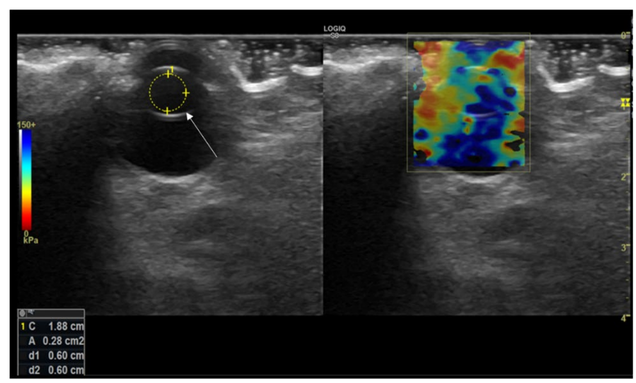

Fig. 2 On the B-mode image, the white solid arrow points at the circular ROI manually selected by the operator

The 2D-SWE was performed on the same day by two operators experienced in performing ultrasound: one with 4 years of expertise in elastography (O1) and one with 2 years of expertise in routine clinical US examinations (O2). Both eyes of the dogs were examined in the same way, the left eye was examined first in all cases. On average, each eye was examined for approximately 5 min. A total of three measurements were taken on three different elastograms using two distinct films and the mean was calculated to plot the results of each eye. All data were acquired in real-time.

Statistical analysis

Statistical analysis was performed with IBM SPSS statistic software (version 23 software for Windows, IBM Corporation, Armonk, NY, USA). The normal data distribution was assessed with Shapiro Wilk test, and data were expressed as mean ± standard deviation; Wilcoxon test was applied to compare different groups of data, considering a P value < 0.05 as statistically significant.

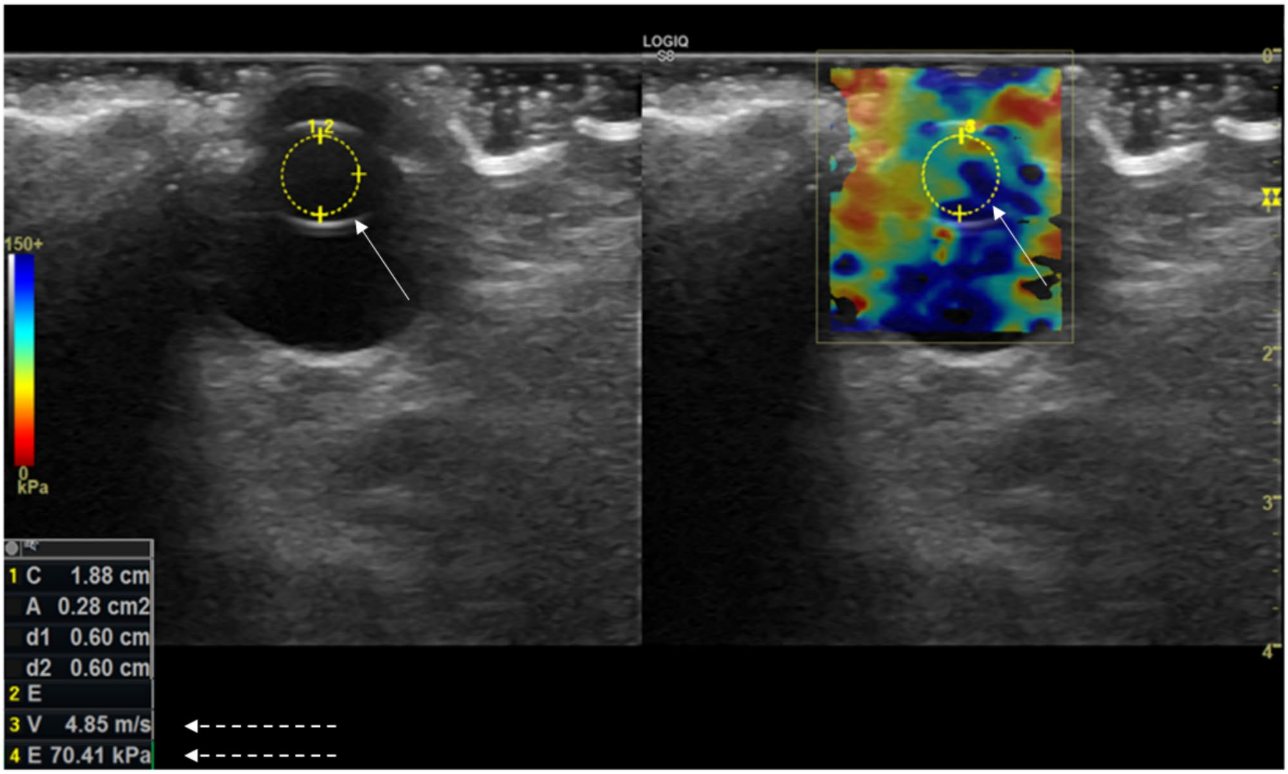

Fig. 3 The same circular ROI pointed by the white solid arrow on the B-mode image is transposed by the system on the colormap on the right side of the image. The final output is the collection of the quantitative stiffness measurements expressed in both m/s and kPa (white dotted arrows)

Then repeatability and reproducibility were assessed through the coefficient of variability (CV%) and intraclass correlation coefficient (ICC).

CV% was computed as a measurement of variability and data dispersion and it was also standardized (sCV%) considering the total dispersion of the studied sample according to the following formula sCV% = CV% / CV%TOT x 100; this was aimed to assess which measurement was more reproducible.

CV% was considered as acceptably low if < 12% [33].

Since on each lens 3 measurements were collected by the operators, three paired comparisons were computed, that multiplied this by 33 dogs, got to 99 paired comparisons used to calculate CV and ICC.

The agreement between measurements collected by the single operator and between the measurements collected by both operators was assessed through intraclass correlation coefficient (ICC) and classified using the following scale: 0.00–0.20 = poor; 0.20–0.40 = fair; 0.40–075 = good; >0.75 = excellent [34, 35].

Abbreviations

NS	Nuclear sclerosis
US	Ultrasound
2D	SWE 2D shear wave elastography
SE	Strain elastography
ARFI	Acoustic radiation force impulse
SWS	Sshear waves
FOV	Field of view
ICC	Intraclass correlation coefficient
CV/sCV	Coefficient of variation-standardized coefficient of variation

Author contributions

Conceptualization, GA, FDS and MV; methodology, FDS, MV; software, FDS; validation, GA, FDS; formal analysis, FDS, SP.; investigation, FDS, SP; data curation, GA, FDS; writing—original draft preparation, GA, FDS, RT, FS, MR, ADB, MV.; writing—review and editing, GA, RT, MR, FS, ADB, SP, FDS and MV; supervision, FDS, MV.; project administration, MV. All authors have read and agreed to the published version of the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

The project has been approved by the CEISA Ethical Committee (Committee on Animal Research and Ethics of the Universities of Chieti-Pescara, Teramo, 360 L'Aquila and of the Experimental Zooprophylactic Institute of Abruzzo-Molise, Prot. N. 12/2019 361 CEISA). Written informed owner's consent was obtained before starting the procedure.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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