Ultrasound Elastographic Features of the Fetlock Joint Capsule in Horses

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Research Article

Keywords: elasticity, elastosonography, fetlock, joint capsule, horse, osteoarthrosis

Posted Date: January 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-149842/v1

Abstract

Background: Capsulitis leads to the release of inflammatory mediators into the joint causing capsular fibrosis and osteoarthrosis (OA). Strain elastosonography (SE) measures the elasticity of tissue evaluating its strain to an operator-dependent deformation. Aims of the study were to assess the feasibility of SE for evaluating the distal attachment of the joint capsule (DJC) of fore fetlocks in sound horses and in horses with fetlock OA and to evaluate differences in their elastosonographic aspect. After a whole lameness examination, fore fetlocks DJC were assigned to Group S and Group P and examined by two operators using SE. The inter-rater reliability (IRR), the intraclass (intra-CC) and interclass (inter-CC) correlation coefficients were used to compare colour grading scores, repeatability, reproducibility of elasticity index (EI) and strain ratio (SR). The same parameters were compared between groups. Findings were significant for P < 0.05.

Results: Forty-one horses were included, 11 in Group S, 30 in Group P (16 with bilateral OA, 8 left and 6 right OA). IRRs ranged from good to excellent. For transverse and longitudinal scans, the EIs of Group S were 0.57-0.51, SRs were 0.32-0.28. Inter-CC and intra-CC were always > 0.8. In Group P, EI was 1.09-0.89, SRs was 0.78-0.72 with intra-CC and inter-CC >0.57. Significative differences of EI and SR were detected between groups, and between Group S and the affected limb of Group P.

Conclusions: SE is a useful technique for evaluating the DJC, with good repeatability and reproducibility. DJC appears softer in sound horses.

Background

Elastosonography is an ultrasound-based technique that measures tissue stiffness and is used to evaluate the mechanical properties of tissues, providing information complementary to B-mode imaging [1, 2]. Strain elastography (SE) measures the relative strain of one area compared to another, as an external stress is applied by the ultrasound probe. This strain is displayed as an elastogram superimposed on the B-mode image, which allows for a qualitative and semi-quantitative analysis of the target tissue, calculating its elasticity Index (EI) and a Strain Ratio (SR) between the tissue and a reference region that is likely to experience the same degree of stress [3].

Due to the release of inflammatory mediators into the synovial cavity, synovitis and capsulitis have been recognized as clinical features of osteoarthrosis (OA) and as drivers of this process [4]. Moreover, chronic capsulitis and synovitis lead to changes in the tissue composition and increased fibrosis [5].

To the author's knowledge this is the first study assessing the feasibility of SE to evaluate the stiffness of the distal attachment of the fore fetlock joint capsules (DJC) in sound horses and in horses with OA, and to assess whether SE can be used to detect a reduction of elasticity of the DJC. The repeatability and the reproducibility of the technique were assessed, and the appearance of the region using strain elastography in both groups was described.

Materials And Methods

All study procedures were approved by the local Ethical Committee (Prot.11/2019). Horse owners were aware of the procedure that were going to be undertaken and signed an informed consent. Adult horses of both sexes and different breeds underwent a whole orthopaedic evaluation by a certified equine surgeon. Horses free from lameness, undergoing a radiographic and ultrasonographic examination of fore fetlocks, were included in group S. Horses that after a lameness examination, had a positive low ring block and/or intraarticular anaesthesia of the metacarpophalangeal joint were included in group P. After the localization of the lameness, radiographic and ultrasonographic examination of both fore fetlocks were undertaken in group P [6].

For diagnostic imaging, all horses were then sedated with xylazine 0.5 mg/kg (Nerfasin, ATI) intravenously. Fore fetlock joints were radiographically and ultrasonographically examined to assess the presence of abnormal findings of osseous or soft tissues structures [7].

Radiographic examinations were performed with a M.T. Medical Technology CS01MS equipment in latero-medial and dorso-palmar views; for the ultrasonographic exams a high frequency linear probe (8.5–10 MHz) connected to an ultrasound system (Logiq S8XD Clear, GE) was used.

Elastography of the DJC at the dorsal aspect P1 was performed by two experienced operators in transverse and longitudinal views with the limb in a weight-bearing position. Elastographic images were independently and randomly analysed by two observers who were blinded to the group the horses were assigned to. Qualitative and semi-quantitative methods were used for evaluation of the elastograms [8]. The Region of Interest (ROI) (20 mm diameter) was placed over the DJC for the qualitative assessment and over the dorsal digital extensor tendon (DDET) as a reference region for the semi-quantitative assessment. For the qualitative analysis, a categorical scoring assessment of DJC was performed, assigning a to DJC a score ranging from 1 to 5 (1 = blue; 2 = green; 3 = yellow; 4 = orange; 5 = red) [9]. where blue encoded for hard and red for soft DJC. For the semi-quantitative assessment, the elastosonographic software calculated the EI of the DJC and the SR between the DJC and the DDET.

To assess intra-operator agreement, every measurement was taken three times by each operator and the data were analysed for normality using the Shapiro-Wilk test. For qualitative analysis, inter-operator agreement on the same image was calculated using the inter-rater reliability (IRR) test [10]. For the semi-quantitative analysis, Mann Whitney U test and Friedman test were used to compare data collected by two operators or by the same operator, respectively. Wilcoxon test compared left to right limbs. The interclass correlation coefficient (inter-CC) and intraclass correlation coefficient (intra-CC) were also calculated for EI and SR for both groups. Colour grading score, EI and SR were compared among Group S and Group P with Mann Whitney U test.

Data were collected on digital worksheets (Excel, Microsoft) and analysed with a statistical software (SPSS V.25.0, IBM). Statistical significance was determined by P values < 0.05.

Results

Forty-one horses were included in the study, with a median age of 10 years (2–20 years); 25 were males (7 stallions, 18 geldings), 16 females.

Eleven horses were assigned to Group S. They were represented by 7 males (2 stallions, 5 geldings) and 4 females, with median age of 8.5 years (5–20 years). They were 4 eventers, 4 western riding horses, and 3 hacking horses.

Thirty horses were allocated to Group P. Sixteen of them showed bilateral involvement, 8 left and 6 right fore-fetlock OA. Eighteen were males (5 stallions, 13 geldings), and 12 females with median age of 10 years (2–20 years). Thirteen were western riding horses, 8 eventers, 3 hacking horses, 3 endurance horses, 2 trotters and 1 racehorse.

Qualitative analysis

In Group S, IRR was excellent for both the transverse and longitudinal views (0.8 and 0.85, respectively), with mean values for the categorical scoring assessment of the DJC of 4.79 and 4.95, respectively.

In Group P, IRR was classified as good in transverse and longitudinal scan (0.56 and 0.59 respectively), with mean values of the categorical assessment of the DJC of 3.6 and 3.8, respectively. In Group P, the affected limbs had a color grading score of 3.68 and 3.78 in transverse scan and longitudinal scan, while the not affected limb had 3.75 and 3.84 ($P < 0.05$).

Color grading score of Group S was significantly higher than the fetlocks of Group P, affected and not affected, in both scans (Table n. 1; Figs. 1A-1B; Figs. 2A-2B) (($P < 0.05$).

Semi-quantitative analysis

In Group S mean EI was 0.57 ± 0.19 (range 0.3–1.3; median 0.5) in the transverse scan, and 0.51 ± 0.14 (range 0.3–2; median 0.6) in the longitudinal scan (Table n.2; Fig. 3A-3B).

Mean SRs were 0.32 ± 0.19 (range 0.1–0.8; median 0.3) and 0.28 ± 0.2 (range 0.2–1.2; median 0.5) in the transverse and longitudinal scans, respectively (Table n.2; Figs. 3A-3B).

In Group S, no statistical difference was observed between or within operators ($P > 0.05$; Mann-Whitney U test and Friedman test). Inter-CC for EI in transverse and longitudinal scan were 0.94, for SR 0.84–0.92. Intra-CC for EI in transverse and longitudinal scan were. 0.98 – 0.97, for SR 0.98 – 0.95 (Table n.3).

In Group P mean EI in the transverse scan was 1.09 ± 1 (range 0.3–5.5; median 0.6) and in the longitudinal scan was 0.89 ± 0.7 (range 0.3–4.1; median 0.6) (Table n.2; Fig. 4A-4B); mean SRs were 0.78 ± 1.48(range 0.1–1.36; median 0.4) and 0.72 ± 0.85(range 0.1–5.2; median 0.4) in the transverse and longitudinal scans, respectively (Table n.2; Figs. 4A-4B).

In Group P, the affected limbs had a mean EI value of 1.05 ± 0.95 (range 0.3–4.9; median 0.6) and 0.83 ± 0.67 (range 0.3–4.1; median 0.6) in transverse and longitudinal scan, while the not affected limbs had a mean EI value of 0.85 ± 0.91 (range 0.3–5.5; median 0.6) and 0.77 ± 0.62 (range 0.4–3.5; median 0.6) (Table n.2).

In Group P, the affected limbs had a mean SR value of 0.7 ± 1.05 (range 0.1–7.1; median 0.4) and 0.68 ± 0.72 (range 0.1-7.0; median 0.4) respectively in transverse and longitudinal scan, while the not affected limbs had a mean SR value of 0.71 ± 1.87 (range 0.1–1.36; median 0.3) and 0.57 ± 0.67 (range 0.1–4.1; median 0.3) (Table n. 2).

Statistical differences were neither observed between or within operators ($P > 0.05$; Mann-Whitney U test and Friedman test).

In Group P the inter-CCs for EI in transverse and longitudinal scan were 0.57-0-94, for SR 0.69–0.92. Intra-CC for EI in transverse and longitudinal scan were 0.94, for SR 0.81–0.96 (Table n.3).

Significative differences of EI and SR values were detected between Group S and Group P (Mann Whitney U test; $P < 0.05$) in both scans (Table n.2). When Group S was compared to the affected limb of Group P, EI and SR were significative lower for Group S (EI 0.57 vs 1.05 in transverse scan and 0.51 vs 0.83 in longitudinal scan; SR 0.32 vs 0.7 in transverse scan and 0.28 vs 0.68 in longitudinal scan) (Mann Whitney U test; $P < 0.05$ (Table n.2).

When Group S was compared to the not affected limb of Group P, EI and SR values were significantly lower in Group S only in longitudinal scan (EI 0.51 vs 0.77; SR 0.28 vs 0.57) (Mann Whitney U test; $P < 0.05$) (Table n.2).

No differences were detected for EI and SR values between the affected and not affected limbs in Group P (Wilcoxon test; $P > 0.05$)

No statistical differences between the right and left limbs were observed in either group ($P > 0.05$; Wilcoxon test). No significant differences were observed between female and male animals ($P > 0.05$; Mann-Whitney U test).

Discussion

Chronic inflammation of the articular capsule is recognized as one of the primary mechanisms of cartilage damage, change in tissue elasticity and cause of fibrosis [4]. Since elastography determines tissue elasticity by measuring the degree of strain that tissues experience in response to external stress [3], we aimed to prove if SE was a feasible technique for evaluating the DJC in sound horses (Group S) and horses with osteoarthrosis (Group P).

No significant difference was found between measures taken on the same structure, by two different operators or between repeated measures from the same operator ($P > 0.05$). But looking at IRR and ICC,

sound horses had better reproducibility and repeatability compared to those of Group P, with higher values of both parameters; horses with OA had a higher variability of the elastographic pattern of the DJC, probably due to different stages of the pathologic process in these joints.

The qualitative appearance of the DJC was slightly softer in the longitudinal scans than in the transverse scans (4.95 vs 4.79 in Group S and 3.78 vs 3.68 in Group P). The longitudinal scans also showed more reliability, potentially due to the anisotropy of musculoskeletal tissues or the increased number of artefacts on the lateral and medial sides of the transverse ultrasonographic image [10–12]. To reduce this variability, the probe was held perpendicularly to the tissues and the lateral and medial aspects of the US images were avoided when selecting the ROI. Finally, as previously described in studies of tendon elastography in humans, it was determined that the EIs and SRs were lower for longitudinal scans than in transverse scans [11].

For the qualitative analysis a significant difference was detected between Group S and Group P when these were considered as a whole sample ($P < 0.05$), and when Group S was compared to the affected limbs and to the not affected limbs of Group P. We suppose that the difference between the Group S and the not affected limb of the Group P can be ascribed to subclinical degenerative changes in the contralateral "sound" joint of this group. However, the lack of further information (MRI, arthroscopy, histology) makes difficult to confirm this theory. Most of all a significative difference was detected between the affected and not affected limb of Group P ($P < 0.05$), suggesting different elastic features between affected and not affected capsule on the same horse.

Concerning the semi-quantitative analysis, EI and SR values were significantly lower in Group S than in Group P. This difference was significative in both scans when Group S was compared to the affected limb of Group P, but only in longitudinal view when Group S was compared to the not affected limb of Group P.

The DDET was chosen as the reference tissue because it was expected to experience the same degree of strain. The ROI was consistently placed at the level of the fetlock articular space in order to facilitate similar comparison of the target region with the same area of the DDET. However, the lack of elastographic data about the DDET could be responsible for the high standard deviation that was observed.

The choice of the ROI is critical for successful SE, especially when large structures are examined or when no clear lesions are visible on B-mode ultrasonography. In this study, only a small ROI was required. In cases where larger structures are examined, care should be taken when analysing results and a computerized analysis of the percentage of pixel distribution may be needed [13].

Even though mild sedation for research purposes was provided to all patients, this may not be necessary in clinical practice when horses are calm and standing still. Standoff pads were not used because they may modify the elastographic appearance of examined tissues and increase artefact occurrence [8].

We successfully evaluated the distal insertion of the fetlock joint capsule in mature horses of different breeds. The technique was feasible in both groups of horses, sound and suffering from fetlock OA, although higher variability of data was found in patients with radiographic and ultrasonographic signs of OA. We also demonstrated that sound horses had an elastogram suggestive of softer DJC compared to the other group.

Although the procedures were not overly technical, reproducibility of the techniques for scanning tissues, reading elastograms, and storing data should be evaluated in less skilled operators. Further studies should investigate the effects of age and breed on the appearance of this anatomical landmark.

Conclusions

This study showed that strain elastography is a technique useful for evaluating the distal insertion of the fetlock joint capsule in horses. We think that it can be used to as complementary diagnostic technique to B-mode ultrasonography to monitor response to treatment or the evolution of rehabilitation programs, in joints suffering from OA. It showed good repeatability and reproducibility and high reliability, especially in qualitative assessments. Future studies should focus on the establishment of a relationship between different degree of osteoarthrosis and elastographic features of the DJC in horses.

List Of Abbreviations

DDET: dorsal digital extensor tendon

DIJ: distal interphalangeal joint

EI: elasticity index

Inter-CC: interclass correlation coefficient

Intra-CC: intraclass correlation coefficient

IRR: Inter-rater reliability

OA: osteoartrosis

P1: first phalanx

ROI: region of interest

SE: Strain Elastography

SR: strain ratio

Declarations

Ethics approval and consent to participate: all the study procedures were approved by the local Ethical Committee (Prot.11/2019) CEISA (Comitato Etico Interistituzionale per la Sperimentazione Animale dell'Università di Chieti e Pescara); an informed consent was provided to the owners providing detailed information about all the study procedures; all methods were carried out in accordance with relevant guidelines and regulations; the study was carried out in compliance with the ARRIVE guidelines

Consent for publication: not applicable

Availability of data and materials: the datasets used and /or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest: the authors declare that they have no competing interests

Funding: not applicable

Authors contribution: PS, GG and AP contributed to acquisition, analysis and interpretation of data; PDF and VV contributed in methodology acquisition and validation of the methods; MV and LP supervised the procedures; all authors substantially revised and approved the submitted version of the manuscript and agreed to be personally accountable for the authors' own contributions. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors would like to thank Abigail Rose Trachtman, DVM, and Ph.D. for English editing of the manuscript.

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Tables

Table 1

color grading score of Group S and of the affected and not affected limb of Group S and P.

	Transverse scan	**Longitudinal scan**
Group S	4.79[a]	4.95[a]
Group P	3.6 [bc]	3.8[bc]
Affected limb in Group P	3.68[c]	3.78[c]
Not affected limb in Group P	3.75[c]	3.84[c]
Different letters on the same column indicate significantly different results (a, b, c: P < 0.05)		

Table 2

Mean EI and SR values for Group S, Group P, for the affected and not affected limb of Group P. Values are expressed as mean ± sd, minimum and maximum value (EI: Elasticity Index; SR: Strain Ratio; sd: standard deviation of the mean).

	EI		**SR**	
	TRANSVERSE	**LONGITUDINAL**	**TRANSVERSE**	**LONGITUDINAL**
Group S	0.57 ± 0.19 [a] (0.3–1.3)	0.51 ± 0.14 [a] (0.3-2)	0.32 ± 0.19 [a] (0.1–0.8)	0.28 ± 0.2 [a] (0.2–1.2)
Group P	1.09 ± 1[b] (0.3–5.5)	0.89 ± 0.7 [b] (0.3–4.1)	0.78 ± 1.48 [b] (0.1–13.6)	0.72 ± 0.85 [b] (0.1–5.2)
Affected limb of P	1.05 ± 0.95 [b] (0.3–4.9)	0.83 ± 0.67 [b] (0.3–4.1)	0.7 ± 1.05 [b] (0.1–7.1)	0.68 ± 0.72 [b] (0.1-7)
Not affected limb of P	0.85 ± 0.91[ab] (0.3–5.5)	0.62 ± 0.62 [c] (0.1-7)	0.71 ± 1.87[ab] (0.1–13.6)	0.57 ± 0.67 [c] (0.1–4.1)
Different letters on the same column indicate significantly different results (a, b, c: P < 0.05)				

Table 3
Inter-CC and intra-CC of EI and SR in transverse and longitudinal scans for Group S and Group P (inter-CC: interclass correlation coefficient; intra-CC: intraclass correlation coefficient; EI: elasticity index; SR: strain ratio).

		TRANSVERSE SCAN	LONGITUDINAL SCAN
Group S	Inter-CC of EI	0.94	0.94
	Intra-CC of EI	0.98	0.97
	Inter-CC of SR	0.84	0.92
	Intra-CC of SR	0.98	0.95
Group P	Inter-CC of EI	0.57	0.94
	Intra-CC of EI	0.94	0.94
	Inter-CC of SR	0.69	0.92
	Intra-CC of SR	0.81	0.96

Figures

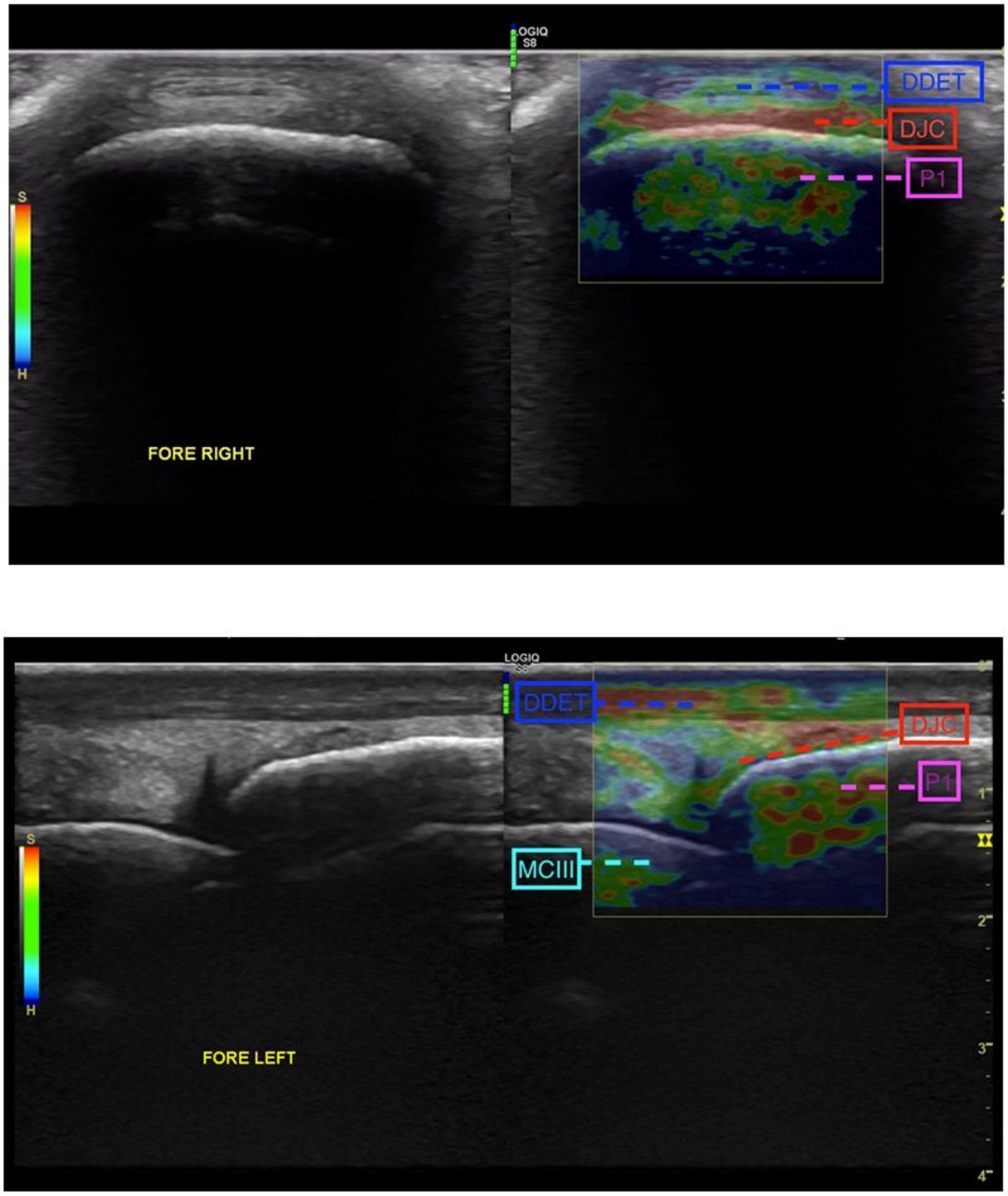

Figure 1

1A–1B: Transverse and longitudinal scans of the DJC of Group S. The elastogram superimposed on the B mode images shows a reddish coloration of the DJC on the transverse image and a green one on the longitudinal scan (red dotted line) (P1: first phalanx; McIII: third metacarpal bone; DDET: dorsal digital extensor tendon; DJC: distal attachment of the joint capsule).

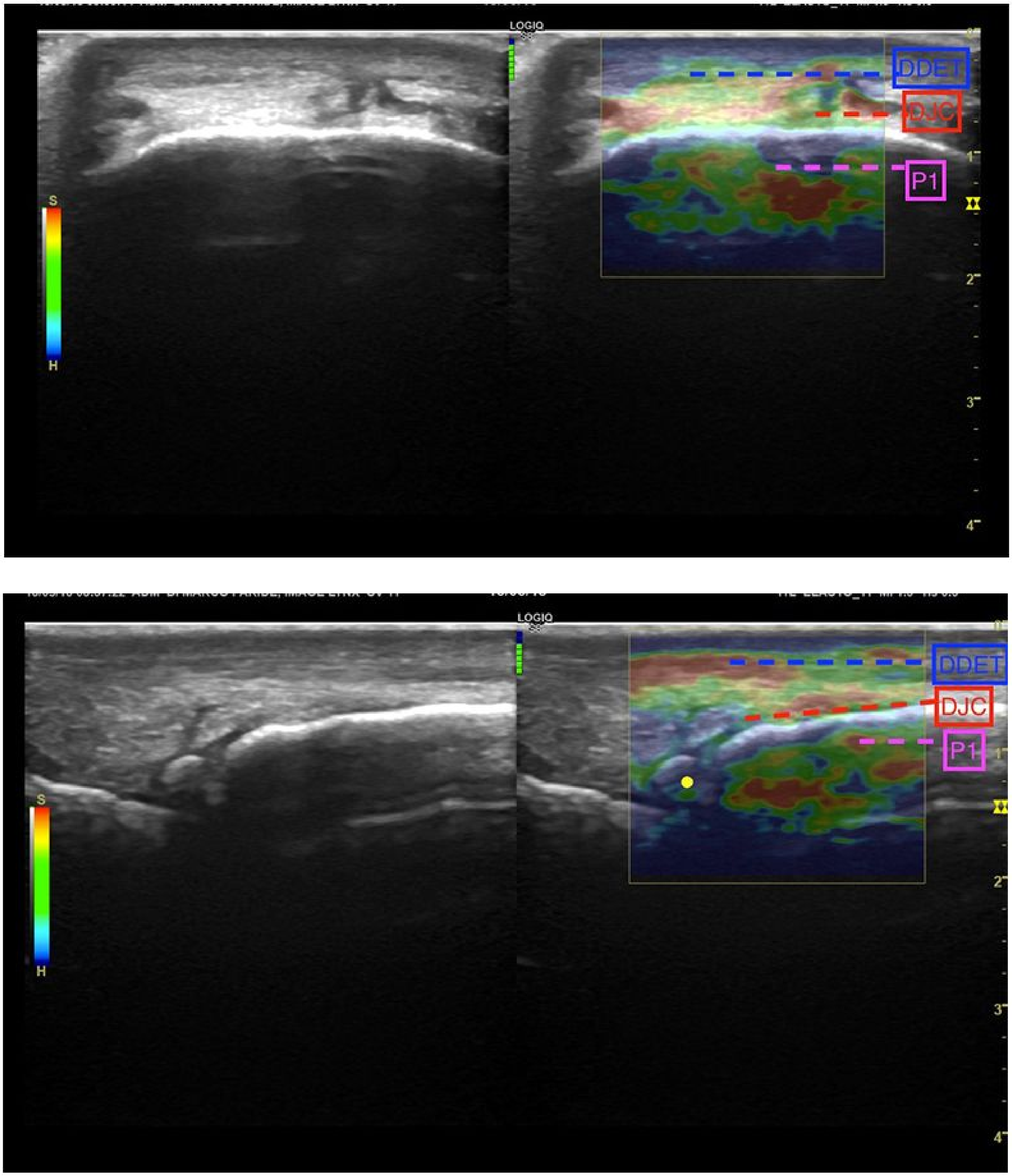

Figure 2

2A–2B: Transverse and longitudinal scans of the DJC of Group P. The elastogram superimposed on the B mode images shows a green coloration of the DJC on the transverse image and green-blue on the longitudinal image (red dotted line) (P1: first phalanx; McIII: third metacarpal bone; DDET: dorsal digital extensor tendon; DJC: distal attachment of the joint capsule). On the longitudinal scan a dorsal fragment is visible (yellow dot)

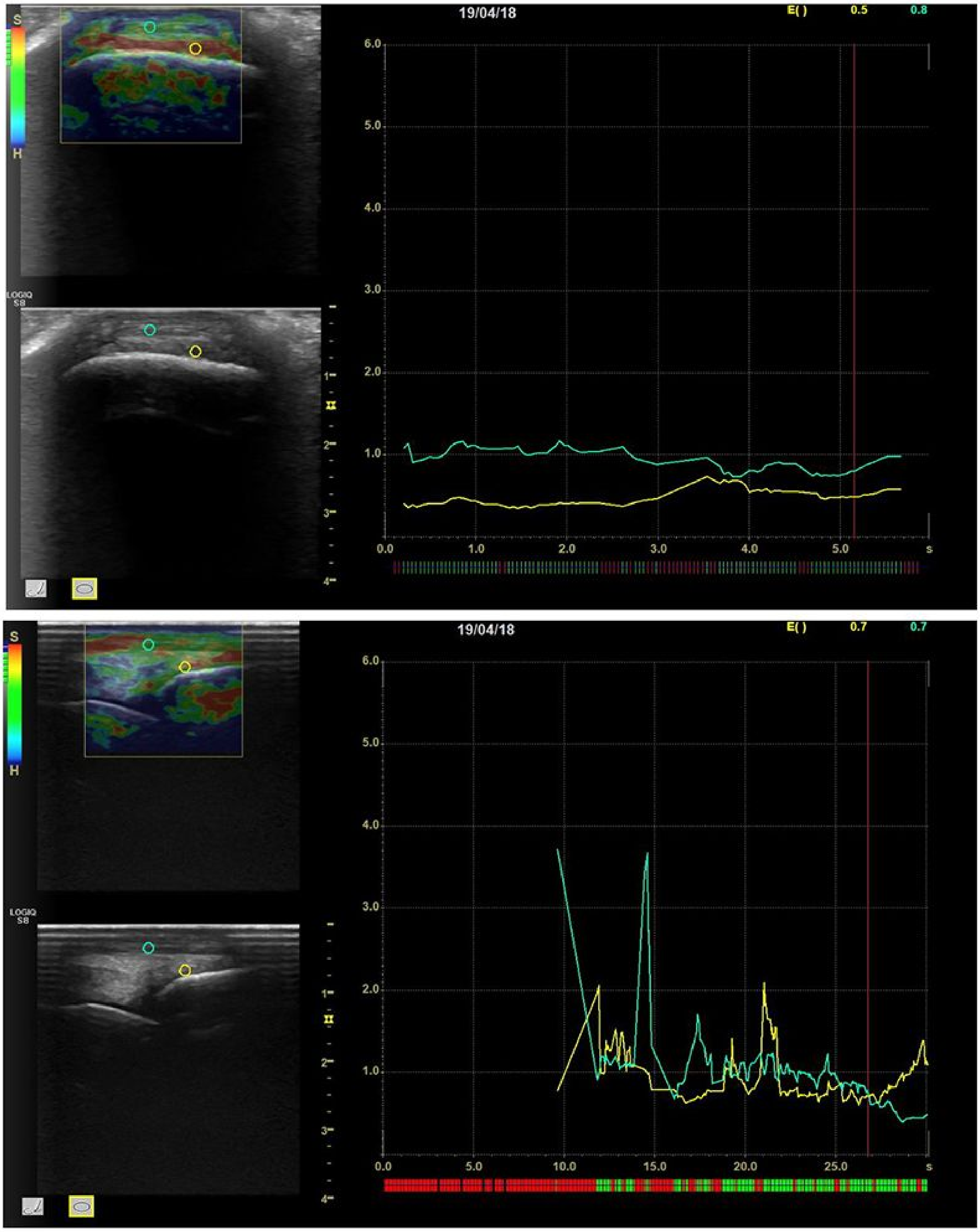

Figure 3

3A-3B: Image showing the colour grading score assigned to the DJC belonging to Group S for qualitative analysis, the EI, and the SR of the DJC in transverse and longitudinal scans (DJC: distal attachment of the joint capsule; EI: elasticity index; SR: strain ratio).

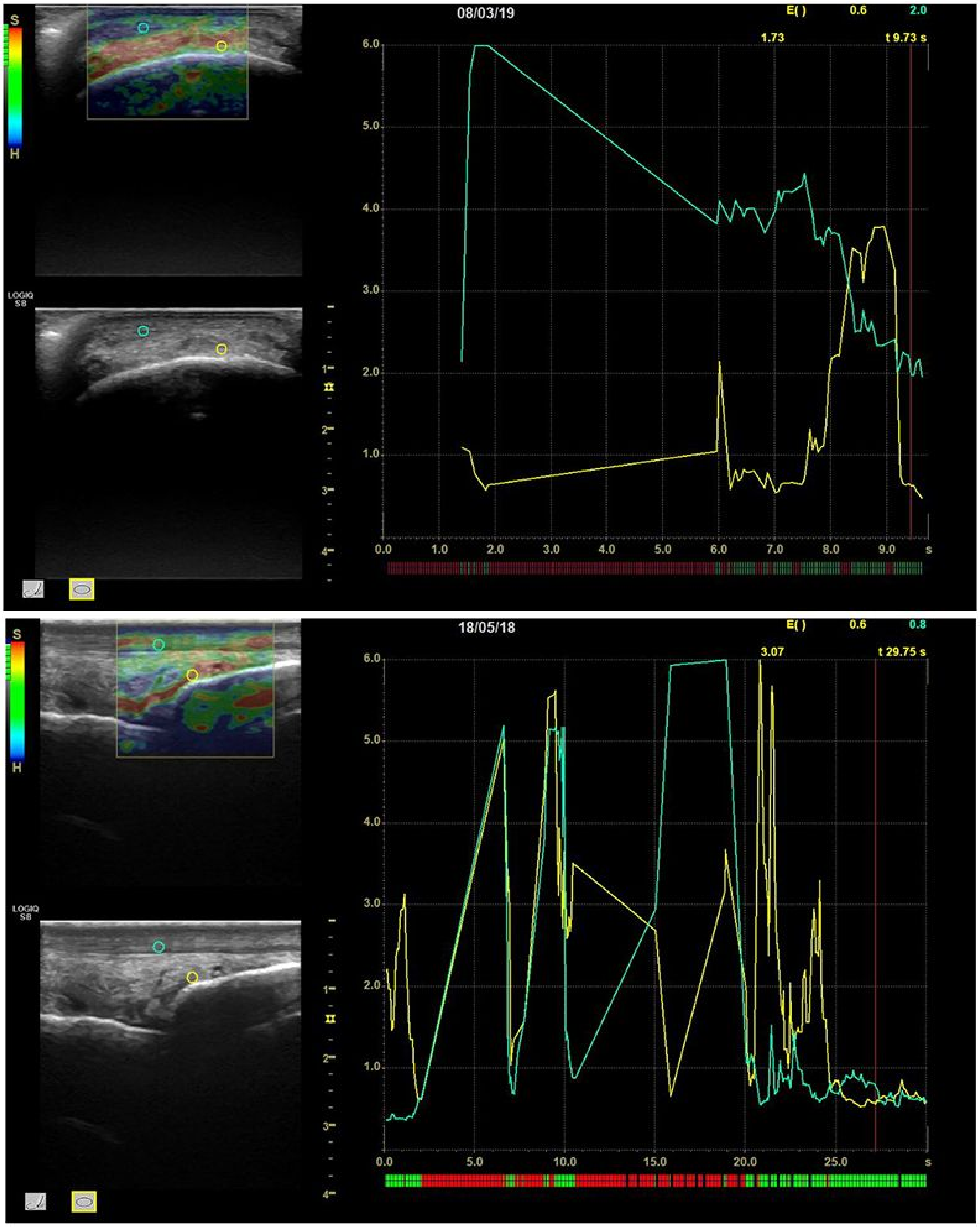

Figure 4

4A–4B: Elastograms of the transverse and longitudinal scans showing the ROIs over the DJC (yellow circle) and the DDET (green circle) in Group P (ROI: region of interest; DJC: distal insertion of the joint capsule; DDET: dorsal digital extensor tendon).