RELIABILITY OF A NEW SEMI-AUTOMATIC IMAGE ANALYSIS METHOD FOR EVALUATING THE DOPPLER SIGNAL AND INTRATENDINOUS VASCULAR RESISTANCE IN PATELLAR TENDINOPATHY

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ABSTRACT

The aim of this study was to study the intra e inter-rater reliability of a new semi-automatic image analysis method for the quantification of the shape of the Doppler signal and the intratendinous vascular resistance in patellar tendinopathy.Thirty athletes (27.4 years; SD: 8.57 years) with patellar intratendinous vascularity were included in a cross-sectional study (42 tendons analysed). The intratendinous blood flow was assessed by Power Doppler and the quantification ImageJ software (v.1.50b) over manual selected ROI. Two blinded observers perform the analysis of the Doppler signal (~~vascular index~~ vascular resistance) and shape descriptors (number of signals, pixel intensity, area, perimeter, major diameter, minor diameter, circularity and solidity). The intraclass correlation coefficient (ICC) was calculated also the Bland-Altman's mean of differences (MoD) and limits of agreement (LOA) were determined. Also, small real differences (SRD) and standard error of measurement (SEM) was calculated. The intra-rater reliability was maximum for area (ICC=0.999; 95%CI=0.998-0.999) and minimum for solidity (ICC=0.782; 95%CI: 0.682 - 0.853). The MoD and the LoA were very low and the relatively SRD and SEM were below 5.3% and 2% respectively. The inter-rater reliability was maximum ICC was for area (ICC= 0.993; 95%CI=0.989-0.996) and the minimum for circularity (ICC= 0.73; 95%CI=0.611-0.817). The MoD and the LoA were low, with SRD and SEM below of 6% and 2.2%. The reliability of the proposed quantitative method to study the intratendinous Doppler signal in patellar tendon is reliability and reproducibility.

Keywords: Ultrasonography, Doppler; Tendinopathy; Image Processing, Computer-Assisted; Vascular Resistance; Blood Flow Velocity

INTRODUCTION

The use of Doppler ultrasound is considered to be especially interesting in the evaluation of tendinopathies (De Jonge et al. 2014; de Vos et al. 2007) because it enables areas with increased blood flow to be observed, and even quantified (Roth et al. 2019; Vlist et al. 2020). Power Doppler facilitates visualisation of low velocity blood flow in very small vessels, representing an effective imaging modality to evaluate intratendinous vascularization (de Vos et al. 2007; Quack et al. 2020).

It is widely accepted that the presence of an intratendinous Doppler signal (DS) can be considered a sign of abnormality in the tendon (Alfredson and Ohberg 2005; Richards et al. 2005), while the absence of such a signal is a sign of healthy tendons (Alfredson et al. 2003; Ohberg et al. 2001). However, these findings contrast with those of other studies that suggest that intratendinous flow is not always a sign of a pathological disorder, but rather a part of an adaptive response to a normal physiological load (Boesen et al. 2012; Malliaras et al. 2008; Tol et al. 2012). Such variability can lead to the study of intratendinous vascularization to be unreliable.

In any case, to quantify a DS, semi-quantitative procedures are frequently used, based mainly on counting scales for grading the degree of DS presence (Simon et al. 2021; Vlist et al. 2020), with limited usefulness because only qualitative data can be obtained. By contrast, quantitative procedures mainly involve colour pixel measurements (Boesen et al. 2012; Koenig et al. 2007b; Terslev et al. 2003b), vessel length (Cook et al. 2005) or, in the case of the resistance index (RI), on automatic ultrasound measurements (Albrecht et al. 2008; Karzis et al. 2017; Koenig et al. 2007b). In addition, the procedures permit the measurement of vascular resistance (VR), which could be useful in assessing the state of the tissue.

In this way it is possible to express numerically tissue resistance to the flow originated by the microvascular bed distal to the measurement site by reference to the RI (Pourcelot and Société parisienne d'expansion chimique 1982) defined as [peak systolic flow - end diastolic flow] / peak systolic velocity. A low RI is associated with low peripheral resistance and high perfusion of the distal bed and, therefore, with an inflammation situation (Bjordal et al. 2006; Koenig et al. 2007a; Terabayashi et al. 2014; Terslev et al. 2003b; Torp-Pedersen et al. 2008).The measurement of RI in intratendinous vessels is complicated because the DS that appear can be very small and numerous, making it difficult or impossible to use the traditional measurement methodology(Koenig et al. 2007a; Koenig et al. 2007b; Terslev et al. 2003a).

Therefore, in the present study, our objective was to study the intra e inter-rater reliability of a new semi-automatic image analysis method for the quantification of the shape of the DS and the intratendinous VR, obtained from pixel intensities (Delorme et al. 1995), that allows quantification on regions of interest (ROI) with numerous and small Doppler signals.

MATERIALS AND METHODS

Study design and participants

For this cross-sectional observational study, a total of 30 athletes (8 women and 22 men) with patellar intratendinous vascularity were included in the study (42 tendons analysed). The age range was between 18 and 50 years old (27.4 years [SD: 8.57 years]) and the participants were voluntarily recruited from a private Physical Therapy Centre (xxx, Spain) in July and August 2018. All participants were informed of the study aims and signed an informed consent document. The study was approved by the Ethical Committee of the XXXX (30/11/2018 CE111803).

Power Doppler parameters and scan method.

The examination was performed with a Telemed SmartUS ultrasound system (Vilnius, Lithuania) and a 7-15 MHz linear probe (L15-7L40H-5). The intratendinous blood flow was assessed by Power Doppler set at a Doppler frequency of 6.7 MHz and 0.7 kHz pulse repetition frequency. The lowest wall filter and gain standardized to just below the level that produced random noise was applied. The adjustment parameters were the same for all patients and pressure on the tendon from the probe was minimized to prevent vessel compression placing the transducer on the skin without pressure.

The patient was positioned in a supine position with the knees extended to avoid occlusion of the vessels due to the tension of the fibres of the patellar tendon(Koenig et al. 2007b) and both knees were evaluated. The patellar tendon was scanned in power Doppler mode in the longitudinal plane at the location of maximum intratendinous Doppler activity and a 4-second video was recorded for further analysis. All the scans were performed by the same ultrasonographist with more than 20 years of experience in musculoskeletal ultrasonography.

Quantification of intratendinous Doppler signal shapes

Processing and analysis of the videos and images was carried out using ImageJ software (v.1.50b). After scaling the image, two observers manually selected and extracted the ROI on the images with the highest and lowest signal corresponding to the systolic peak and at the end of diastole for each patient. The image data were coded, anonymized and randomized thereafter to avoid possible bias or recall effects.

The observers, who were blinded to the patient's data, analysed the set of the images at two different times with at least a 15 days delay.

Since the DS appears in colour on a grayscale background, it is easy to segment and isolate the region for quantification. We used the colour threshold plugin, which allows the cut-off point to be adjusted manually with slider bars.

To quantify the DS, the saved frame with the highest DS from each video was selected and the area of colour pixels was calculated. In addition, the number of signals, pixel intensity, area, perimeter, major diameter, minor diameter, circularity, and solidity were automatically calculated on the frames with the highest DS of each recording (figure 1). Circularity and solidity are dimensionless parameters included in the so-called shape descriptors that evaluate the shape of a contour. When circularity is about 1, the contour is like a circle and when is about 0 is like a line. The solidity is a ratio that indicates the relation between the area of the shape and the convex area (theorical maximum =1)

Quantification of intratendinous vascular resistance

The flow pattern was evaluated by calculating the mean pixel colour of the DS for each image. The pixel colour mean of the image with the highest signal was considered as the maximum systolic velocity, and the one with the lowest signal as the final diastolic velocity. These data were transferred to the RI formula, giving a value associated with the intratendinous VR (Figure 1).

In the images in which a DS was not detected , or did not present an intratendinous DS in diastole, the VR was considered as 1, which represents normality in the musculoskeletal tissue (Koenig et al. 2007a; Terslev et al. 2003c).

Statistical analysis

As the sample size allowed a normal distribution to be assumed, parametric tests were applied for all the variables, and the descriptive statistics used to summarize the data for each of the evaluators were mean, standard deviation, range, and quartiles. The analyses were conducted for the number of DS, intensity of colour, total area (mm2) of active vessels, total perimeter (mm) of active vessels, major diameter, minor diameter, circularity (index between 0 and 1), solidity and VR.

The intraclass correlation coefficient (ICC) was calculated based on a total agreement and two-factor random-effects model (ICC2,1) for each of the variables of interest(McGraw and Wong 1996; Weir 2005). This coefficient offers values of between 0 and 1, where 0 would be a lack of agreement and 1 would be total agreement. Although the interpretation of these cut-off points is, to a certain extent, arbitrary, in our context an ICC above 0.90 was considered excellent, between 0.90 and 0.75 as good, between 0.75 and 0.50 as moderate and below 0.50 as poor (Portney and Watkins 2009).

Measurement precision(Atkinson and Nevill 1998; Lexell and Downham 2005) was evaluated using the standard error of measurement (SEm) [SEm=SD•√(1 ICC)] and its relative value with respect to the average of all measurements and the smallest real difference (SRD). SRD is useful for determining whether a change in the parameter is due to a real change or lies within the limits of error of the measuring method [SRD=1.96•SEm•√2](Schuck and Zwingmann 2003).

The limits of agreement (LOA) were calculated according to the method described by Bland and Altman(Bland and Altman 1986; Hopkins 2000) and the presence of summative or multiplicative biases with Passing-Bablock's linear regression method(Bablok et al. 1988; Passing and Bablok 1983). For a direct clinical interpretation, the graphical method proposed by Luiz et al.(Luiz et al. 2003), based on the Kaplan-Meier estimate representing the probability of survival as a function of the degree of disagreement, was applied.

The analyses were conducted using IBM SPSS Statistics 19.0 (SPSS Inc. IBM Company, 2010) and the jmv package (version 0.9) for R (version 3.5.0; 2018).

RESULTS

Patient characteristics

Thirty participants aged between 18 and 50 years (27.4 years [SD: 8.57 years]) took part in the study. All of them presented intratendinous vascularity: 22 (73%) symptomatic and 8 (27%) asymptomatic. Twelve participants presented bilateral and eighteen unilateral intratendinous vascularization, meaning that a total of 42 tendons were analysed. In addition, each image was analysed at maximum systolic speed and minimum diastolic speed so that finally all the parameters were calculated by both observers for a total of 84 images (table 1).

Reliability

Overall, both intra- and inter-rater reliability was very good, and no additive or multiplicative biases were detected (Supplementary Figure 1 and Supplementary Figure 2).

More specifically, the intra-rater ICC was maximum for area (ICC=0.999; 95%CI=0.998 - 0.999) and minimum for solidity (ICC=0.782; 95%CI: 0.682 - 0.853). The MoD and the LoA (Table 2 and Figure 2) were very low with respect to the magnitude of the measurement (at least one order), and the relatively small real differences were below 5.3% and relative SEM (%) below 2% (for VR). Suplementary Figure 3 depicts the plot obtained with the Kapplan-Meier method. Although it cannot be taken as an indicator of reproducibility, no differences were found in t-test for mean differences.

The agreement was also very good for inter-rater reliability, although, as expected, slightly lower than the intra-rater reliability (Table 3 and Figure 3). Similarly, the maximum ICC was for area (ICC= 0.993; 95% CI=0.989-0.996) and the minimum for circularity (ICC= 0.73; 95%CI=0.611-0.817). The MoD and the LoA remained at least one order below the measurement, with a relative SEM (%) below of 2.2% and with a relative SRD (%) below of 6% in the number of signals. Suplementary Figure 4 of shows a practical interpretation of the magnitude of the differences.

DISCUSSION

Our results demonstrate very good intra- and inter-observer reliability both for measurements of the DS and for the calculation of intratratendon VR. These good results are probably due to the semi-automatic nature of the measurement procedure, in which the dependence of the operator is only involved in the selection of the location of the intratendinous ROI, in the adjustment of the parameters of the computer program for the selection of the signal Doppler and in the detection of images with higher and lower Doppler signal.

In comparing our results with those of other studies, we can only focus on the quantification of the DS area because VR can only be quantified using the RI. This is because the quantification methods that have been used were mainly based on the number of coloured pixels(Ellegaard et al. 2008; Strunk et al. 2007) or on semi-quantitative scales corresponding to the count of the number of DS(D'agostino et al. 2009; Risch et al. 2018; Sunding et al. 2016), while RI measurements are made automatically through the ultrasound scanner(Albrecht et al. 2008; Qvistgaard et al. 2001; Terslev et al. 2003b).

The rest of the morphological and pixel intensity variables of the DS studied here obtained good intra-rater and inter-rate reliability results, although we have not found studies that have examined the reliability of these variables, making it impossible to compare results.

In patients with rheumatoid arthritis (Qvistgaard et al. 2001), the area of the DS in the synovium have been quantified to determine the degree of joint inflammation of the fingers, the methodology used presenting excellent intra-rater (ICC:0.82-0.97 ; $p<0.0001$) and good inter-rater (ICC; 0.81; $p <0.0001$) reliability. In the study of tumour vascularization in patients with gestational trophoblastic neoplasia, the intra-rater reliability was also excellent (ICC = 0.94) (Li et al. 2018). These good results coincide with our study, in which quantification of the area showed excellent agreement for intra-rater and inter-rater reliability. Such good results are possible because is a semi-automatic procedure whereby the influence of the operator is minimal.

Counting the number of the intratendinous DS by the investigator is essential for the different evaluation scales of the DS to be applied. Its use in the presence of abnormalities in the quadriceps tendon, patellar and Achilles tendons and plantar fascia(Bandinelli et al. 2011), led to excellent results for intra-rater (ICC: 0.97; 95%CI: 0.90-1) and inter-rater (ICC: 0.95; 95%CI: 0.89-1) reliability, using the classification of D´Agostino et al.(D’agostino et al. 2009). Poltawski et al. evaluated reliability to quantify hyperaemia in the common extensor tendon in tennis elbow(Poltawski et al. 2012). To evaluate the DS, they used a PD scale that assigned five grades based on a subjective estimation of the extent of visible blood vessels. Inter-rater reliability was good (ICC: 0.89; 95% CI: 0.79–0.95) for DS graduation Sunding et al. analysed the intra and inter-rater reliability of evaluating Achilles and patellar tendon neovascularization by means of colour Doppler using a modified Öhberg score(Sunding et al. 2016). The intra-rater reliability results were good for neovascularization measured with this qualitative scale in the patellar tendon (kappa coefficient = 0.79-0.86) and the Achilles tendon (kappa coefficient = 0.64-0.78). However, the inter-rater reliability results were moderate for neovascularization in the patellar tendon (kappa coefficient = 0.45-0.76) and the Achilles tendon (kappa coefficient = 0.59-0.87).

Although in our method the intratendinous ROI must be selected manually to determine the number of DS, this is more sensitive than a visual inspection for detecting the signals, and both intra-rater and inter-rater reliability scores are good and clearly better than what can be achieved using qualitative methods. Qualitative methodology seems sensitive to slight changes in the number of vessels when complex vascularization is scored(Risch et al. 2018). However, this scoring procedure allows easy, immediate and absolute quantification of the intratendinous vessels and, therefore, may be suitable for application in clinical practice.

Another method to quantify the vascularization of the patellar tendon is that proposed by Cook et al. using colour Doppler and measuring the length of the vessels(Cook et al. 2005). The test-retest reliability of the measured vessel length was excellent [ICC: 0.94; 95%CI: 0.88–0.97), with good raters [ICC 0.84; 95CI: 0.51–0.94). However, unlike our quantification system, this classification system does not provide information on vascular diameter, and it remains debatable whether evaluation of the total vessel length is relevant to clinical practice(Cook et al. 2005; Risch et al. 2016).

In the few studies that have measured intratendinous VR, the most commonly used methods are based on the automatic measurement of RI using the pulsed Doppler mode, either as an average of three vessels (Koenig et al. 2007a; Koenig et al. 2007b), or for a single vessel (Karzis et al. 2017). We have found no studies that have tested the reliability of intratendinous RI measurements, probably because many authors consider that does not depend on the experience of the researcher, but on the ultrasound machine itself (Terslev et al. 2003b). However, the reliability of RI measurements has been tested in other tissues, by measuring the IR of a single vessel. Albrecht et al. evaluated inter-rater reliability of IR (ICC: 0.60) in hand and wrist arthritis during anti-inflammatory treatment (Albrecht et al. 2008). Strunk et al. conducted a similar study, evaluating only the wrist (Strunk et al. 2007), they found a weak correlation between observers (r-Pearson = 0.53).

. The test-retest reliability of assessments of clitoral blood flow in healthy women using colour Doppler in a pelvic floor muscle contraction task obtained moderate to good intra-rater reliability for the RI at rest (ICC: 0.67; 95%CI: 0.08-0.88; $p = 0.018$) and excellent reliability after a pelvic floor muscle contraction task (ICC: 0.81, 95%CI: 0.51-0.92; $P < 0.001$) (Mercier et al. 2018). These RI reliability results are poorer than the intratendon VR measurements used in our study (ICC = 0.921; 95% CI = 0.859-0.957), which may be due not only to the influence of the operator or the technical equipment, both of which influence the acquisition and interpretation of the images (Albrecht et al. 2007; Patil and Dasgupta 2012), but also to the difficult localization of the same intra-articular blood vessel (which can be very small) during the examinations, whereas our study used an the mean of all the SD found in the ROI.

In the analysed studies, the reliability results for the RI are worse than those obtained for quantification of the area of DS, perhaps because the results shown for the RI include those obtained during the exploration process of each researcher, and also because the RI is a relationship that depends on several measurements at different times. Intuitively, parameters that depend on several measurements are more likely to have lower reliability, compounded by the imperfect reliability of the individual measurements included in their equations (Mercier et al. 2018). This difference contrasts with our results, in which the VR shows excellent intra and inter-rater reliability, coincides with the results of most of the Doppler area quantification variables, probably due to the semi-automatic nature of the quantification procedure in both cases.

The main limitation of this study is that, although the reliability of the method is good, the analysis must be performed offline the ultrasound device and requires extra time for video export, image extraction and image analysis and, therefore, its clinical application is not immediate. However, it can be considered a valid method for use in the investigation and quantification of tendon vascularization in this context.

The other limitation is related to the determination of the RI, which, although it could be obtained from spectral analysis, in this study we have calculated it indirectly from the pixel intensity in systole and diastole. The advantage of this approach is that the intensity values are obtained using the same procedure as other morphological parameters, thus saving time. However, it would be interesting in a future study to test the correlation between the resistance index RI obtained in this way and that obtained by spectral analysis.

In future studies, it would also be interesting to analyse the colour Doppler mode, which is currently gaining momentum for evaluating intratendon vascularization, due to the improved sensitivity of ultrasounds scanners(Torp-Pedersen et al. 2015).

Furthermore, including an examination of patients could give results more typical of clinical practice. Finally, the shape variables of the Doppler signal and the VR could be correlated with the symptoms of the patient and their prognostic and monitoring capacity could be analysed.

This quantification methodology shows very good reliability and reproducibility and is capable of combining the quantification of the number of signals, the magnitude and the VR of the tissue, which would allow a more precise evaluation of the state of the tissue, the improved monitoring of changes over time and the establishment of a threshold between pathological and physiological blood flow.

CONCLUSION

The results obtained confirm that the proposed method has very good reliability and reproducibility, while any influence on the detected DS is negligible. In this sense, it will be of interest to extend the study to ascertain the reliability between different ultrasound scanners and software, which could increase the robustness of the method.

Conflict of Interest: The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article and declare no conflict of interest.

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FIGURES

Figure 1. Quantification of intratendinous Doppler signals and color pixel quantization. Left frame in peak systolic Flow. Right frame in diastolic flow. The analysis method shows de number of Doppler signals and their morphology. The intensity of signals permits to obtain the vascular resistance.

Figure 2. Bland-Altman's plots for intra-observer (A1-A2) agreement. In order: Number of signals, pixel intensity mean, area, perimeter, major and minor diameter, circularity, solidity and vascular resistance

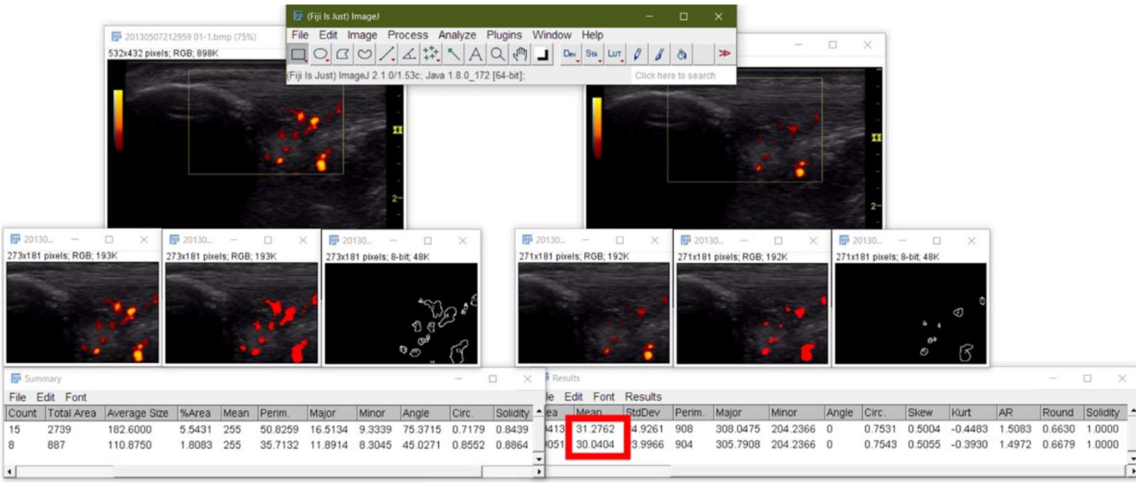
Figure 3. Bland-Altman's plots for inter-observer (A1-A2) agreement. In order: Number of signals, pixel intensity mean, area, perimeter, major and minor diameter, circularity, solidity and vascular resistance.

Suplementary Figure 1. Scatterplots for the relation intra-rater (A1-A2) measurements. In order: Number of signals, pixel intensity mean, area, perimeter, major and minor diameter, circularity, solidity and vascular resistance.

Suplementary Figure 2. Scatterplots for the relation inter-rater (A1-A2) measurements. In order: Number of signals, pixel intensity mean, area, perimeter, major and minor diameter, circularity, solidity and vascular resistance.

Suplementary Figure 3. Intra-observer Kaplan-Meier estimate that represents the probability of survival as a function of the degree of disagreement. The dotted lines represent the minimum differences for 50% of the observations. In order: Number of signals, pixel intensity mean, area, perimeter, major and minor diameter, circularity, solidity and vascular resistance .

Suplementary Figure 4. Inter-observer Kaplan-Meier estimate that represents the probability of survival as a function of the degree of disagreement. The dotted lines represent the minimum differences for 50% of the observations. In order: Number of signals, pixel intensity mean, area, perimeter, major and minor diameter, circularity, solidity and vascular resistance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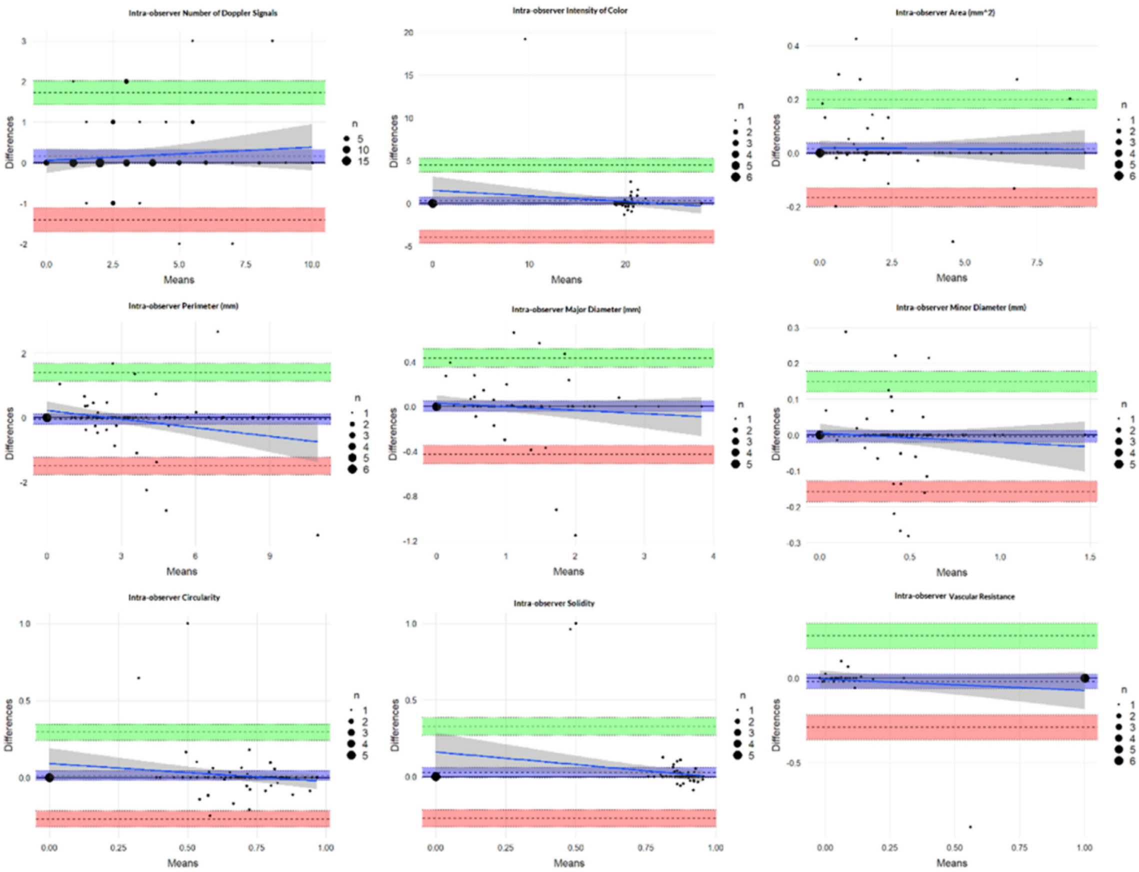
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Intra-observer Intensity of Color
Intra-observer Area (mm^2)
Intra-observer Perimeter (mm)
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Intra-observer Vascular Resistance
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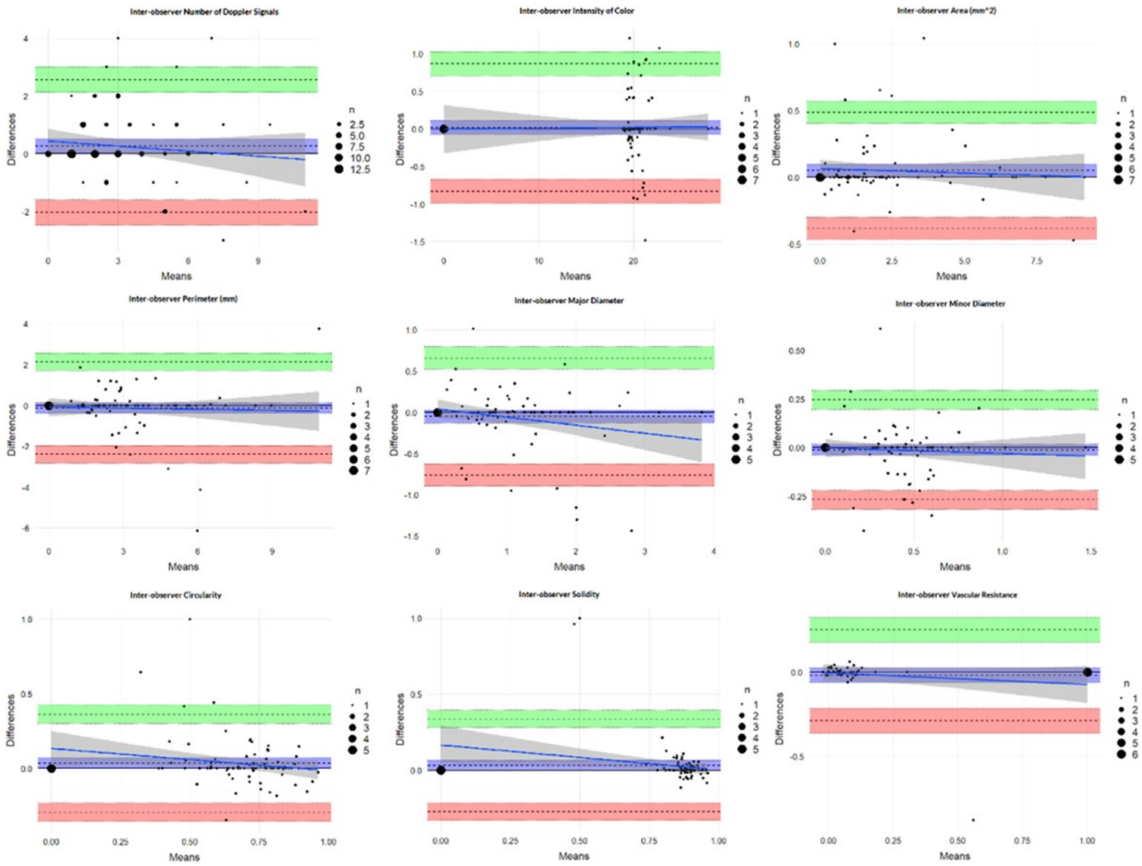
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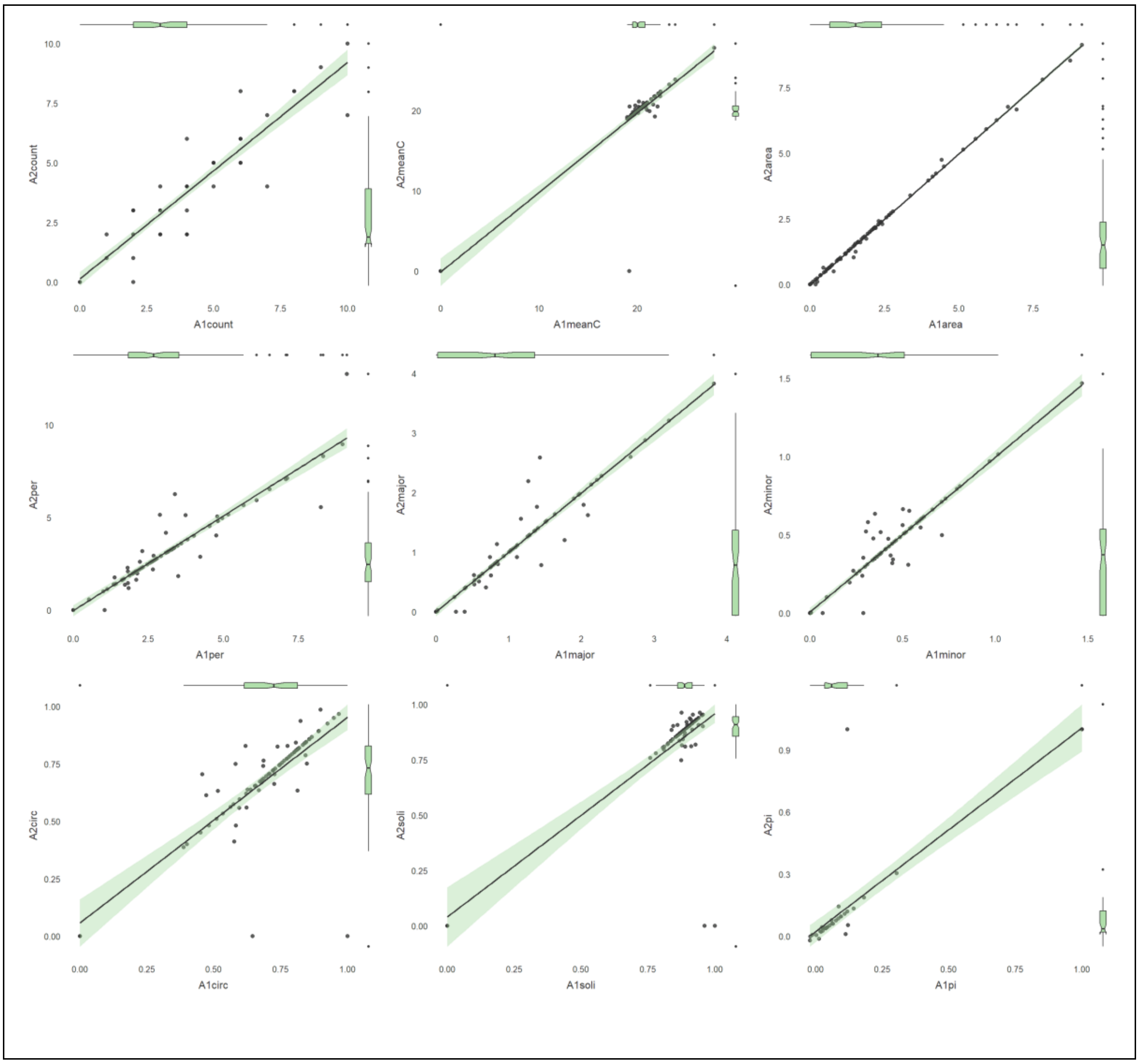
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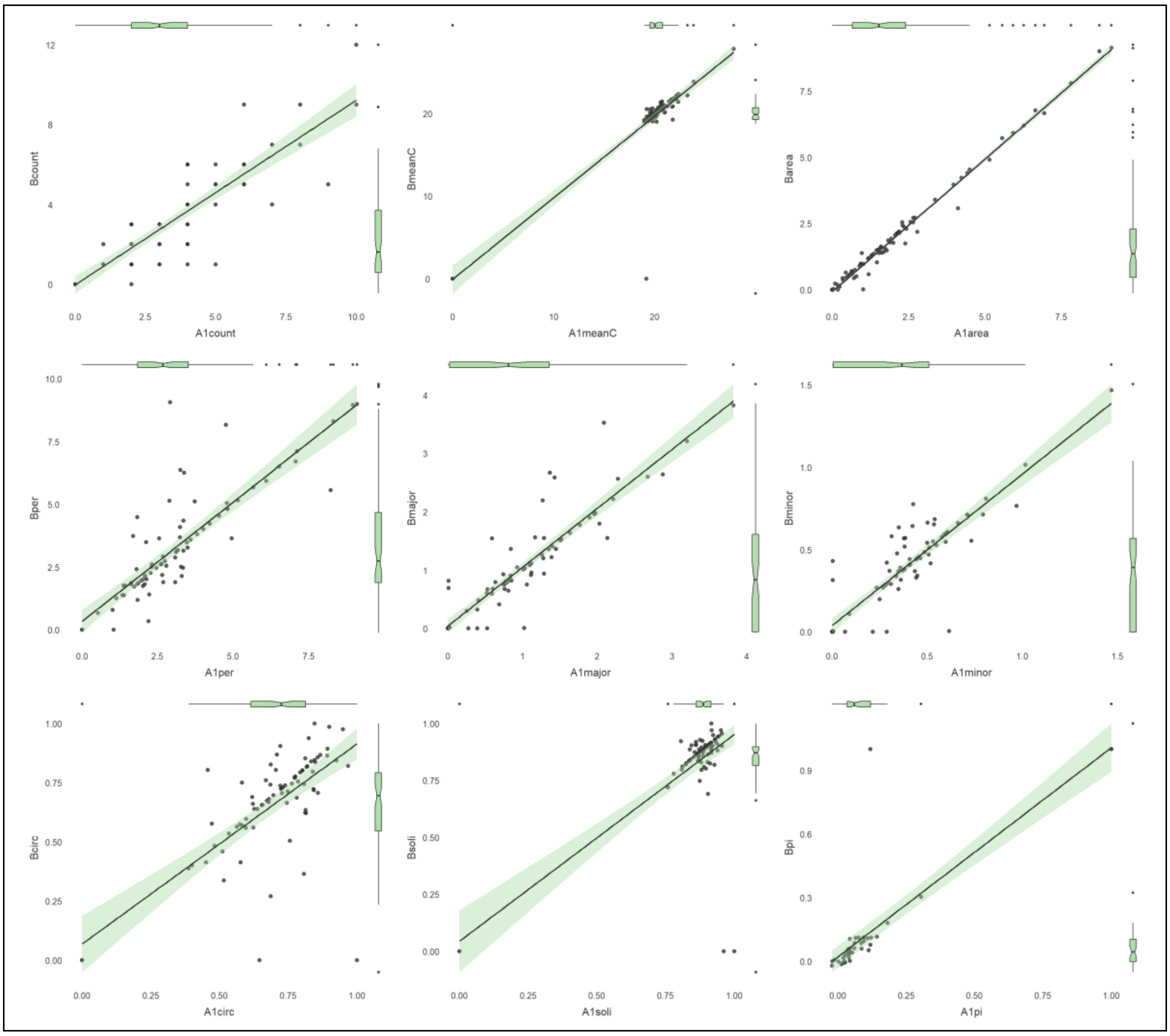

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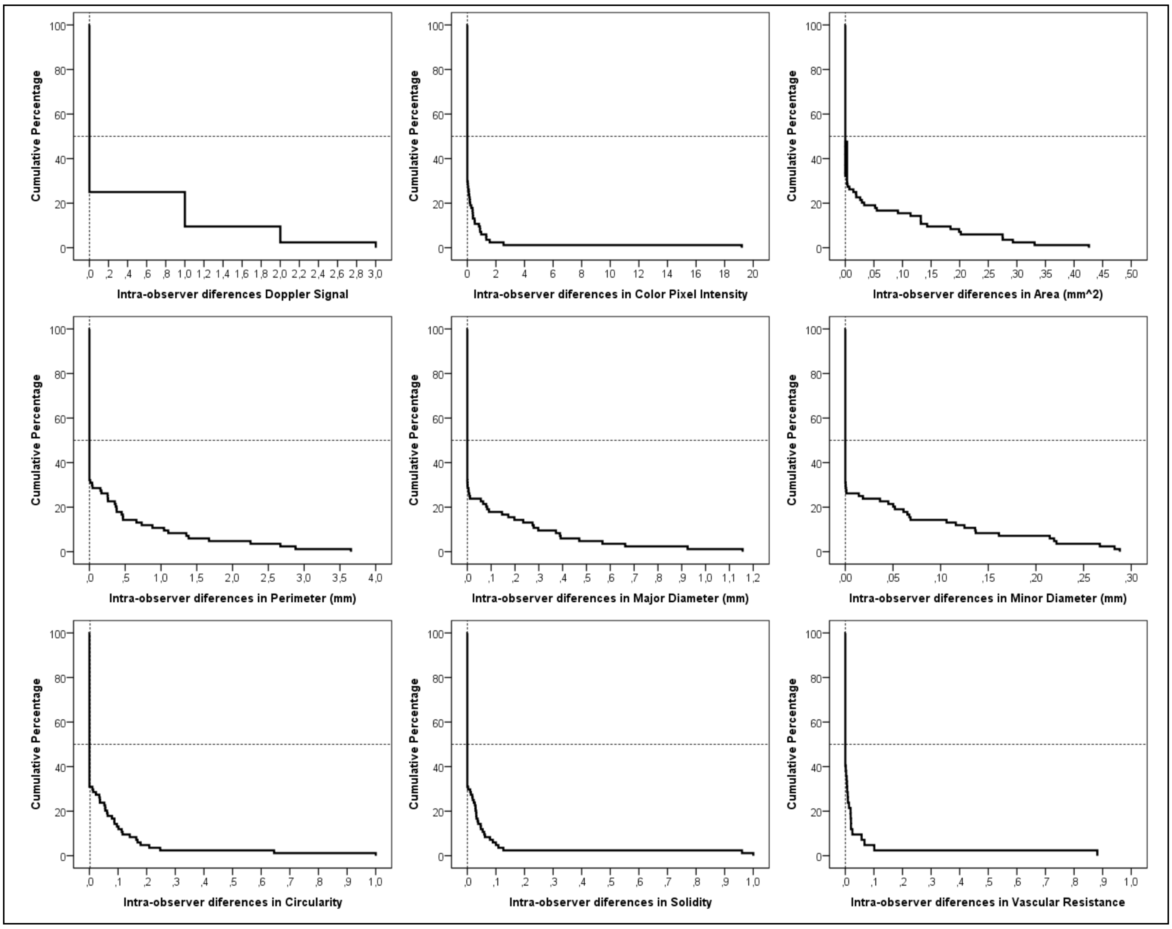
INTRA-OBSERVER



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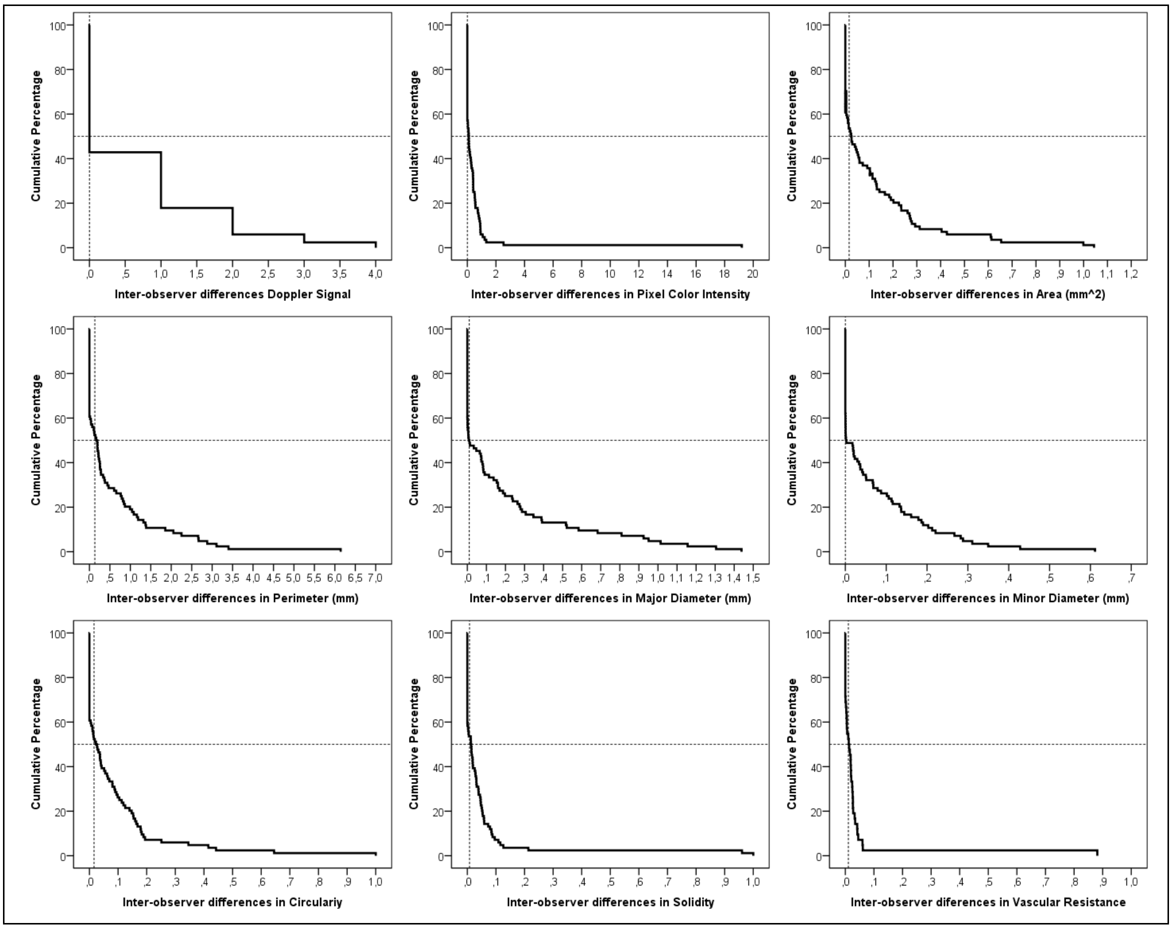

TABLES

Table 1. Baseline characteristics.

Variable (n=30)	Mean (SD)	Min	Q1	Median	Q3	Max
Age	27.4 (8.57)	18	21	23	35.5	50
Time.Evol	21 (26.34)	0	0	12	36	120
VisaP	75.2 (22.18)	29	56.75	75.5	100	100
Tendinopathy; n(%)	22 (73%) Yes					
Side lession; n(%)	12 (40%) Right	10 (33%) Left				
Sex; n(%)	22 (73%) Male	8 (27%) Female				
Sport practice; n (%)	22 (73%) Yes	8 (27%) No				

Data are presented as mean (standard deviation) and range for quantitative variables and counts and percentage for categorical variables. Time.Evol= time of evolution in months. VisaP: Victorian Institute of Sport Assessment – Patella.

Table 2. Intra-observer reproducibility and reliability.

Parameter (n=84)	Mean (SD) 1	Mean (SD) 2	t-value	p-value	Effect	MoD (LoA)	ICC (95%CI)	SEM	SEM%	SRD	SRD%
Number of signals	3.17 (2.281)	3.01 (2.209)	1.78	0.080	0.19	0.16 (-0.02 a 0.33)	0.935 (0.901-0.958)	0.028	0.90%	0.08	2.50%
Pixel intensity (0-255)	18.98 (5.449)	18.71 (5.806)	1.20	0.235	0.13	0.28 (-0.19 a 0.74)	0.928 (0.891-0.952)	0.053	0.28%	0.15	0.78%
Area (mm^2)	2.05 (2.041)	2.04 (2.043)	1.71	0.090	0.19	0.02 (0 a 0.04)	0.999 (0.998-0.999)	0.000	0.02%	0.00	0.05%
Perimeter (mm)	3.04 (1.986)	3.09 (2.165)	-0.61	0.542	0.07	-0.05 (-0.21 a 0.11)	0.937 (0.904-0.958)	0.009	0.29%	0.02	0.80%
Major diameter (mm)	0.918 (0.8263)	0.916 (0.8524)	0.06	0.954	0.01	0 (-0.05 a 0.05)	0.966 (0.948-0.978)	0.000	0.02%	0.00	0.06%
Minor diameter (mm)	0.35 (0.2821)	0.354 (0.2892)	-0.48	0.633	0.05	0 (-0.02 a 0.01)	0.963 (0.944-0.976)	0.001	0.16%	0.00	0.44%
Circularity (0-1)	0.678 (0.2154)	0.665 (0.2393)	0.81	0.419	0.09	0.01 (-0.02 a 0.04)	0.803 (0.712-0.867)	0.004	0.59%	0.01	1.65%
Solidity (0-1)	0.836 (0.2154)	0.808 (0.2493)	1.64	0.105	0.18	0.03 (-0.01 a 0.06)	0.782 (0.682-0.853)	0.009	1.10%	0.03	3.04%
Vascular resistance (n=42)	0.198 (0.3362)	0.218 (0.3582)	-0.95	0.350	0.15	-0.02 (-0.06 a 0.02)	0.921 (0.859-0.957)	0.004	1.92%	0.01	5.31%

SD: standard deviation; Effect: Cohen's *d.* MoD (LoA): mean of differences (95% Limits of agreement); ICC (95% CI): intraclass correlation coefficient (95% Confidence Interval); SEM: standard error of mean; SRD: smallest real difference.

Table 3. Inter-observer reproducibility and reliability.

Paramete (n=84)	Mean (SD) 1	Mean (SD) 2	t-value	p-value	Effect	MoD (LoA)	ICC (95%CI)	SEM	SEM%	SRD	SRD%
Number of signals	3.17 (2.281)	2.9 (2.408)	2.05	0.044	0.22	0.26 (0.01 a 0.52)	0.871 (0.806-0.915)	0.067	2.19%	0.18	6.08%
Pixel intensity (0-255)	18.98 (5.449)	18.69 (5.799)	1.27	0.208	0.14	0.02 (-0.08 a 0.11)	0.927 (0.889-0.952)	0.057	0.30%	0.16	0.84%
Area (mm^2)	2.05 (2.041)	1.99 (2.056)	2.81	0.006	0.31	0.05 (0 a 0.1)	0.993 (0.989-0.996)	0.004	0.20%	0.01	0.54%
Perimeter (mm)	3.04 (1.986)	3.22 (2.203)	-1.50	0.138	0.16	-0.14 (-0.39 a 0.11)	0.85 (0.777-0.9)	0.051	1.64%	0.14	4.54%
Major diameter (mm)	0.918 (0.8263)	0.97 (0.9077)	-1.32	0.190	0.14	-0.05 (-0.13 a 0.03)	0.912 (0.868-0.942)	0.011	1.16%	0.03	3.21%
Minor diameter (mm)	0.35 (0.2821)	0.362 (0.2898)	-0.81	0.422	0.09	-0.01 (-0.04 a 0.02)	0.895 (0.843-0.931)	0.003	0.74%	0.01	2.06%
Circulatiry (0-1)	0.678 (0.2154)	0.642 (0.2454)	1.97	0.053	0.21	0.04 (0 a 0.07)	0.73 (0.611-0.817)	0.013	2.00%	0.04	5.55%
Solidity (0-1)	0.836 (0.2154)	0.803 (0.2491)	1.91	0.060	0.21	0.03 (0 a 0.07)	0.772 (0.667-0.846)	0.011	1.34%	0.03	3.70%
Vascular resistance (n=42)	0.198 (0.3362)	0.217 (0.3593)	-0.88	0.386	0.14	-0.02 (-0.06 a 0.02)	0.921 (0.859-0.957)	0.004	1.78%	0.01	4.95%

SD: standard deviation; Effect: Cohen's *d*. MoD (LoA): mean of differences (95% Limits of agreement); ICC (95% CI): intraclass correlation coefficient (95% Confidence Interval); SEM: standard error of mean; SRD: smallest real difference.