

Validation of New Semi-Automated Ultrasound Image Analysis Method for Assessing Vascular Resistance in Musculoskeletal Tissues

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DOI:

10.32098/mltj.01.2023.04

LEVEL OF EVIDENCE: 6

SUMMARY

Background. The aim of this study was to analyze the concurrent validation between the resistance index (RI) obtained by Spectral Doppler (SD) and the vascular resistance (VR) calculated by quantifying pixel color intensity of the power Doppler (PD) signal.

Methods. The brachial artery of 30 healthy participants (24.8 yrs; SD = 6.44 yrs) were evaluated with SD to automatically obtain RI and with PD to estimate de VR with de Pourcelot's formula from systolic and diastolic peaks. Three assessments were performed on each participant, obtaining a total of 90 ultrasound assessments of the brachial artery with their respective RI.

Processing and analysis were performed ImageJ software were manually selected and extracted from the brachial artery PD images with the highest and lowest signal corresponding to peak systolic and end diastole for each patient. The mean pixel color of the image with the highest signal was considered as the peak systolic velocity and of the image with the lowest signal as the end-diastolic velocity.

Results. A high correlation was found between RI and VR ($r = 0.92$; 95%CI = 0.88 to 0.95; $p \leq 0.001$) so there is a very strong concurrent validity between the two measures, and they can be considered equivalents (common variability of 84%).

Conclusions. This new method of analyzing DS by quantifying the color intensity of the PD signal pixel is a good predictor of RI and could be useful for VR analysis in musculoskeletal tissues where measurement of RI is complicated such in neovascularization in tendinopathies with multiple Doppler signals.

KEY WORDS

Image analysis; musculoskeletal vascularization; Power-Doppler ultrasonography; Resistance Index; vascular resistance.

INTRODUCTION

The use of Doppler ultrasound in the assessment of musculoskeletal pathologies is becoming increasingly common (1, 2). Doppler ultrasound can be used to localize areas of increased blood flow and quantify color pixels, which can provide clinically important information, more accurate diagnoses, and assessments of response to treatment (3). In tendinopathies,

it allows locating areas with intratendinous Doppler signal (IDS) (4), although we must keep in mind that the presence of IDS does not provide relevant data about the tendinopathy (5, 6). However, the study of vascular resistance (VR) could provide more useful information on tissue status (7, 8). Currently, the pathophysiological model of the continuous model of tendinopathy is assumed (9), in which the degenera-

tive and reactive process coexist (10), and although this model makes little reference to inflammation, some studies have observed a coexistence of inflammatory and degenerative processes in the development of tendinopathies (7, 11, 12).

Spectral Doppler allows assessment of the type of blood flow, low or high resistance, by means of a velocity *versus* time curve that represents the variation of red blood cell flow velocity throughout the cardiac cycle. Spectral Doppler can be used to measure the degree of resistance to arterial flow originating from the distal microvascular bed, expressed numerically with the resistance index (RI). On a musculoskeletal level, a RI equal to 1 is considered normal, while a lower value could be associated with inflammatory processes (7, 13, 14). The RI is provided directly by spectral Doppler ultrasound from the Pourcelot formula:

$(\text{peak systolic velocity} - \text{end-diastolic velocity}) / (\text{peak systolic velocity})$ (15).

For this purpose, the Doppler window is adjusted longitudinally, centered on the vessel lumen and occupying 2/3 of its section. IDS seen in tendinopathies may contain vessels with a smaller lumen than the Doppler window, so in many cases it is not possible to adjust the Doppler window to the size of these vessels. In addition, many IDS may appear, complicating and multiplying the measurement process in the attempt to obtain the mean RI of the IDS.

To avoid this problem, some authors have proposed to measure the RI in the three intratendinous vessels with the largest diameter and to calculate the mean (13, 14, 16) but the large number of intratendinous vessels that can be found suggests that this methodology is probably not adequate enough to reproduce the entire intratendinous vascular resistance.

The analysis of the IDS through the color pixels not only allows quantification of the area of the Spectral Doppler (17), but also the establishment of a relationship between pixel color intensity scale and flow velocity (18), so it could be used to measure the intratendinous vascular resistance through the resistance index formula. Contrary to the RI measurement, this procedure would allow its application on any number and size of IDS found within the Doppler window. Although the intra- and inter-observer reliability of selection and quantification in Power Doppler mode has already been demonstrated (19), there are no studies that have analyzed the relationship between the RI provided directly by the ultrasonographic device and that estimated from the intensity of the color pixels. The consistent validity of the latter method would allow its application in tendon hypervascularization.

The aim of this study was to analyze the concurrent validation between the resistance index obtained by spectral Doppler and the vascular resistance calculated by quantifying pixel color intensity of the power Doppler signal.

MATERIALS AND METHODS

Study design and participants

A total of 30 healthy participants with a systolic blood pressure lower than 130 mmHg and diastolic blood pressure lower than 80 mmHg and no medical history of pathologies or medication that could alter blood flow were included for this study. Participants were recruited voluntarily at a private physiotherapy center (Clinica F&C Fisioterapia Avanzada y Neurorehabilitación, Huelma, Spain) in December 2018. All participants were informed about the study and signed an informed consent document. The study was approved by the Ethics Committee of the Catholic University of Murcia (CE111803 – Date of approval: November 30, 2018) and follows the rules of Muscles, Ligaments and Tendons Journal (MLTJ) (20).

Power Doppler and Spectral Doppler evaluation

The patient was placed in supine decubitus with the right upper limb in a comfortable position to image the brachial artery.

The brachial artery was chosen to test the two methods because measurement of the RI in the brachial artery does not offer difficulties, since it is a single vessel with a large lumen diameter and allows automatic acquisition of this index by the ultrasound scanner itself. In addition, the RI of the brachial artery is easily modified by vascular occlusion maintained for 5 min, which will generate hyperemia at 1 min after occlusion, with a decrease in RI (21). In other words, vascular occlusion of the brachial artery allows alteration of the RI recordings, decreasing its value to test the two methods in different situations.

The examination was performed with two Telemed SmartUS ultrasound scanners (Vilnius, Lithuania) with 7-15 MHz linear transducers (L15-7L40H-5) and a Riester Minimum III pressure cuff (Jungingen, Germany) placed on the most distal part of the participant's arm (22-24).

One of the transducers was installed on an articulated arm to maintain its static position on the longitudinal plane of the brachial artery at the midpoint between the antecubital and axillary regions. The ultrasound machine, in spectral Doppler mode, identified the cardiac cycles, as well as the peak systolic velocity and end-diastolic velocity, automatically calculating the RI of the brachial artery.

The second transducer was placed distal to the first and transversal to the brachial artery to record the PD. A Doppler frequency of 6.7 MHz and a pulse repetition frequency of 1.5 kHz were used. The lowest wall filter and standardized gain were applied just below the level that produced random noise. The setting parameters were the same for all patients and minimal pressure was exerted with the transducer on the arm to avoid vessel compression.

Brachial artery occlusion was applied for 5 min distal to the ultrasound slices with the pressure cuff, using a pres-

sure calculated from the systolic arterial pressure +50 mmHg (23, 25). In this way, endothelium-mediated, nitric oxide-dependent ischemia and subsequent reactive hyperemia were induced.

At 1 min post-occlusion and coinciding with the vasodilatation generated by hyperemia and therefore a decrease in the RI (21, 26, 27), the RI and a 4-second video of the transverse PD signal to the brachial artery were recorded by the two ultrasound scanners at the same time. The location of the ultrasound transducers and pressure cuff can be seen in **figure 1**.

Three assessments were performed on each participant, obtaining a total of 90 ultrasound assessments of the brachial artery with their respective RI and 4 seconds videos of the PD signal after vascular occlusion. All scans were performed by the same sonographer with more than 15 years of experience in musculoskeletal ultrasound.

Quantification of intratendinous vascular resistance with Doppler ultrasonography

Processing and analysis of the videos and images were performed by a second investigator using ImageJ software (Version 1.50b, National Institutes of Health, Bethesda, MD, USA). After image scaling, regions of interest (ROI) were manually selected and extracted from the brachial artery PD images with the highest and lowest signal corresponding to peak systolic and end diastole for each patient. Measurement and image data were coded and anonymized to avoid potential bias.



Figure 1. Location of ultrasound transducers and pressure cuff.

Since the IDS appears in color on a grayscale background, it is easy to segment and isolate the region for further quantification. We used the color threshold plug-in, which allows the cutoff point to be manually adjusted with slider bars.

The flow pattern was assessed by calculating the mean pixel color intensity of the previously isolated PD signal for each image. The mean pixel color of the image with the highest signal was considered as the peak systolic velocity and of the image with the lowest signal as the end-diastolic velocity. These data were transferred to the RI formula, giving a value associated with the RV of the brachial artery (**figure 2**).

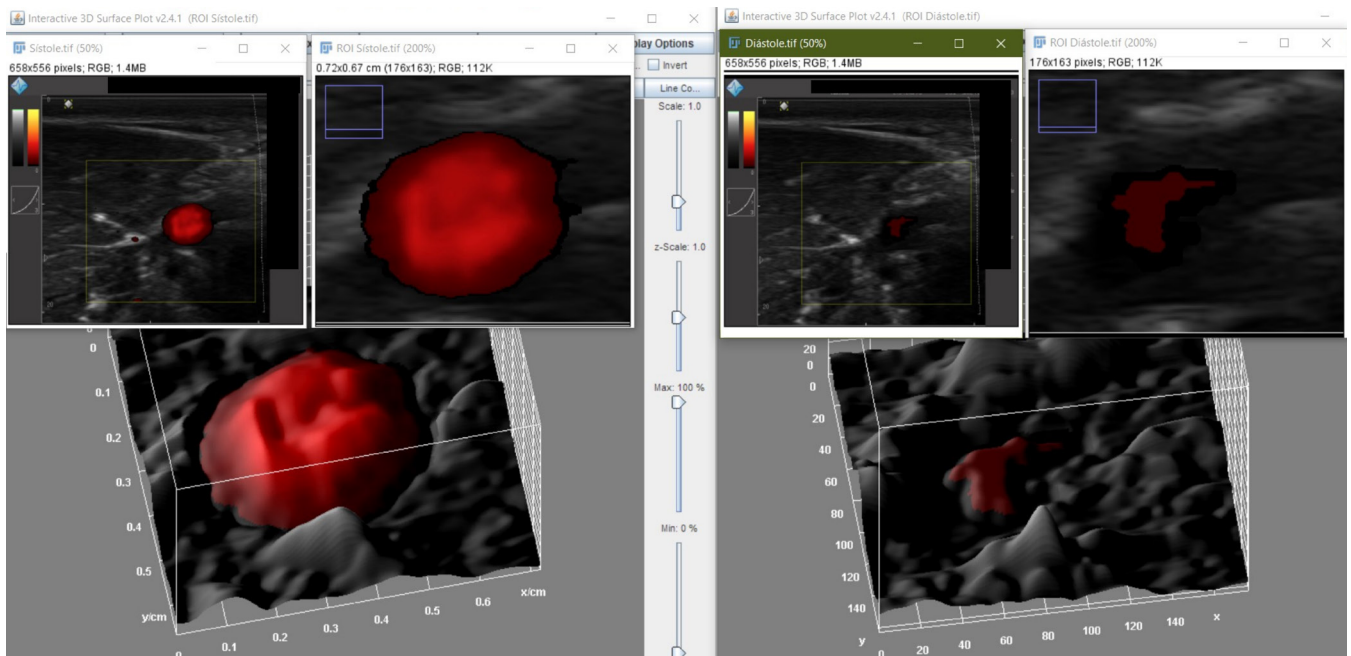


Figure 2. Quantification of vascular resistance by pixel color intensity from Power Doppler video with Doppler signal processing and calculation of mean of mean color pixels.

The reproducibility and reliability of this procedure has been previously studied in IDS (19) with a very good intra- and interobserver agreement (ICC = 1.92; 95%CI = 0.86 to 0.96).

Sample size

This study has an exploratory aim, so a sample of 30 healthy participants were recruited in a non-random, purposive, and consecutive manner.

Statistical methods

Data analysis was conducted with the statistical package R v.3.6 and JASP Team (2020). JASP (Version 0.14.1) (Computer software).

Data were summarized using mean and standard deviation and range and quartiles for continuous variables and absolute and relative frequencies for categorical variables.

Although the sample size allows the assumption of normality, multivariate and bivariate normality was explored with the Shapiro-Wilks test; in all contrasts the W statistic was near to 1.0, so the assumption of normality was finally assumed.

Correlation was assessed with Pearson's r correlation coefficient (95%CI) and the percentage of shared variability between pairs of variables was determined with the coefficient of determination ($r^2\%$). In addition, the Vovk-Sellke Maximum p-Ratio was estimated, which provides the degree of plausibility evidence for the alternative hypothesis (presence of correlation), compared to the null hypothesis (no correlation), regardless of the P-values (28).

As a guideline, the following cut-off points will be considered for correlations: 0.0 to 0.19 – very weak correlation; 0.2 to 0.39 – weak correlation; 0.4 to 0.69 – moderate correlation; 0.7 to 0.89 – strong correlation; 0.9 to 1.0 – very strong correlation. In all cases, the significance level was set at $p \leq 0.05$.

Table I. Descriptive summary of Doppler parameters.

Parameter	Mean	SD	VC%	Min	Q1	Median	Q3	Max
Area SPV	7520.1	1997.56	27%	3656.0	5980.5	7877.5	9037.0	11383.0
Area FDV	2328.4	1222.99	53%	268.0	1493.3	2152.0	3035.5	6356.0
Color pixel intensity SPV	40.1	9.18	23%	25.7	33.5	38.2	47.1	61.8
Color pixel intensity FDV	21.6	2.75	13%	19.0	19.5	20.8	22.7	31.7
Resistance index	0.82	0.073	9%	0.68	0.76	0.84	0.87	0.96
Vascular resistance	0.44	0.115	26%	0.23	0.35	0.44	0.50	0.69

SPV: Systolic Peak velocity; FDV: final diastolic velocity; SD: Standard deviation; VC%: variation coefficient; Q1: quartile 1; Q2: quartile 2; Min: minimum; Max: Maximum.

Table II. Pairwise correlations between PD color variables and RI.

Pairwise correlation	Pearson's r (95%CI)	P-value	R2%	VS-MPR
RI-VR	0.92 (0.88 to 0.95)	≤ 0.001	84%	≥ 300
Systolic Area-SPV.px	0.39 (0.2 to 0.55)	≤ 0.001	15%	262
Systolic Area-FDV.px	-0.018 (-0.37 to 0.03)	0.09	3%	1.7
Systolic Area-RI	0.53 (0.36 to 0.67)	≤ 0.001	28%	≥ 300
Systolic Area-VR	0.57 (0.41 to 0.69)	≤ 0.001	32%	≥ 300
Diastolic Area-SPV.px	0.27 (0.07 to 0.45)	0.010	7%	7.8
Diastolic Area-FDV.px	0.61 (0.47 to 0.73)	≤ 0.001	38%	≥ 300
Diastolic Area-RI	-0.04 (-0.24 to 0.17)	0.073	0%	1.0
Diastolic Area-VR	-0.02 (-0.23 to 0.19)	0.85	0%	1.0
SPV.Px-RI	0.72 (0.61 to 0.81)	≤ 0.001	52%	≥ 300
SPV.Px-VR	0.85 (0.78 to 0.9)	≤ 0.001	72%	≥ 300
FDV.Px-RI	-0.2 (-0.39 to 0.01)	0.06	4%	2.3
FDV.Px-VR	-0.15 (-0.34 to 0.06)	0.16	2%	1.2

RI: resistance index; VR: vascular resistance; SPV.px: mean color pixel intensity in systolic peak of velocity; FDV.px: mean color pixel intensity in final diastolic velocity. Vovk-Sellke Maximum p-Ratio: based on the P-value, the maximum possible odds in favor of H1 over H0 equals $1/e^{-p}$ ($\log(p)$) for $p \leq 0.37$. Values close to 1 indicate no favored H1.

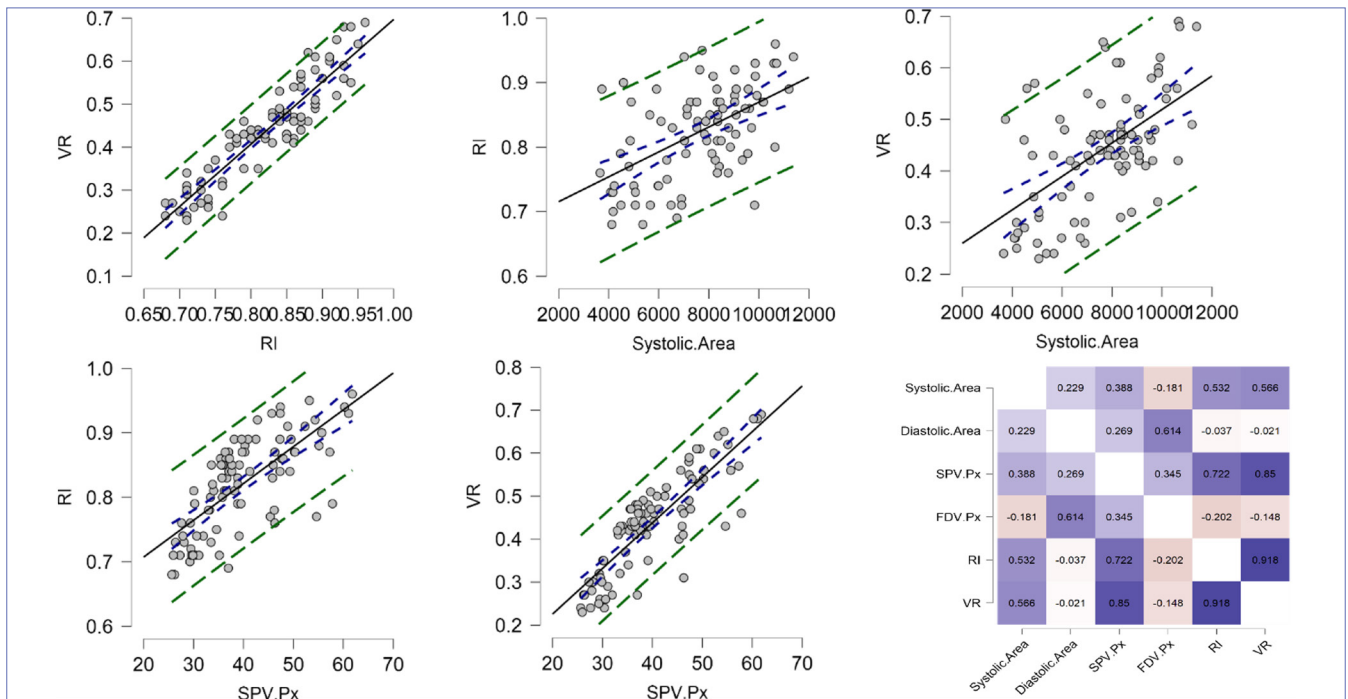


Figure 3. Regression lines between RI, VR, and systolic parameters and Pearson's r heatmap. RI: resistance index; VR: vascular resistance; SPV,px: mean of color pixel in systolic peak velocity.

RESULTS

Thirty participants (16 women (53%), mean age = 24.8 years; SD = 6.44 years) were included in this study. A descriptive summary of variables is shown in **table I**.

The correlations between variables of interest obtained from IDS, RI and VR are shown in **table II**, and **figure 3**.

A high correlation was found between RI and VR ($r = 0.92$; 95%CI = 0.88 to 0.95; $p \leq 0.001$) so there is a very strong concurrent validity between the two measures, and they can be considered equivalents (common variability of 84%).

We found interesting moderate correlations between the Doppler area at the systolic peak with respect to IR ($r = 0.53$; 95%CI = 0.36 to 0.67; $p \leq 0.001$) and RV ($r = 0.57$; 95%CI = 0.41 to 0.69; $p \leq 0.001$) in a similar way, but no correlation with respect to the area in diastole.

Similarly, a strong correlation was found between the mean intensity of color pixels in systole with respect to RI ($r = 0.72$; 95%CI = 0.61 to 0.81; $p \leq 0.001$) and RV ($r = 0.85$; 95%CI = 0.78 to 0.90; $p \leq 0.001$), but not with respect to diastole.

DISCUSSION

No other studies have been found that correlate RI with other methods of measuring VR, so the results obtained cannot be compared. Regarding the correlation between VR calculat-

ed by quantifying the color intensity of the pixels of the PD signal and the RI measured at the same time and over the brachial artery, it was observed that there is a positive correlation, that is, the higher the value of VR, the higher the RI.

It is difficult to think that the mean raw pixel color of the PD signal correlates with the flow velocity, because there are different filtering and interpolarization algorithms to represent the image that can distort the results. Moreover, this study used the PD mode, which encodes the amplitude of the signal by the number of red blood cells that are moving, which provides information on the amplitude of the IDS, as opposed to Color Doppler which encodes the average of the velocities.

This issue could further complicate this correlation because the RI is calculated with flow velocity data and the PD provides information on intensity. Although the results obtained in this study have been very good, it would be interesting to study the correlation of this methodology applied on the color IDS. However, in relation to the RI and given that it is a relative index, it seems that the two types of measures are equivalent. In 1995, Delorme studied the relationship between the mean pixel color of the color Doppler and flow velocity (18). The limitations of this modality of ultrasound are related to the interpolation algorithms used by the ultrasound machine itself when low levels of color Doppler are used, giving erroneous low values for the mean pixel color value.

The selection of an inadequate velocity scale and insonation angle in the color Doppler mode can generate aliasing (29), altering the color representation of the signal pixel. Despite these limitations, the use of average pixel color analysis of the color Doppler is applicable in practice and provides additional information in the assessment of IDS (18).

Although the quantification of the color IDS has been extensively studied (18, 30, 31), quantification of PD signals has several advantages over color Doppler in the assessment of tissue vascularization and blood flow. In the case of this study, the PD mode has been used because it is less dependent on the Doppler angle, it is more sensitive in the representation of low flows in small vessels, aliasing does not appear (32), and the codification in a single-color tone facilitates the quantification process and the results obtained correlate well with the perfusion rate (33).

Limitations

This quantification methodology has some limitations, mainly related to standardization, as it depends on the sensitivity and color tone of the PD signals, which are altered by different parameters such as: the ultrasound model and transducer used, the selection of ultrasound slices, the selection of images with higher and lower IDS from the video sequence, the settings of the ultrasound Doppler parameters, the color scale used, the delimitation of the ROI of the IDS in the image and the manual selection of the color thresholds of the image analysis software. However, in this study all these parameters have been optimized and kept constants for all scans.

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CONCLUSIONS

As conclusion, our method of analyzing intratendinous Doppler signal by quantifying the color intensity of the Power Doppler signal pixel is a good predictor of resistance index and could be useful for vascular resistance analysis in tissues where measurement of resistance index is complicated as in tendinopathies.

FUNDINGS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

JJMP, FMC: images providing. FJMP: videos and images procession and analysis. JRD: data processing and analysis. FJMP, JRD: writing. FJMP and JRD contribute equally to this work. All authors revised and approved the final version of manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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