A preliminary study using virtual touch imaging and quantification for the assessment of skin stiffness in systemic sclerosis

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ABSTRACT

Objective. *To evaluate ultrasound Virtual Touch Imaging and Quantification (VTIQ) as a method for determining absolute skin stiffness in patients with systemic sclerosis (SSc).*

Methods. *Skin thickness, assessed by the modified Rodnan Skin Score (mRSS) and absolute skin stiffness, using VTIQ, were measured at all mRSS anatomical sites to quantify the shear wave velocity (in m/s) in 26 SSc patients and in 17 age- and gender-matched controls. Correlations between mRSS and absolute skin stiffness, and comparisons between patients and controls were analysed statistically using Mann-Whitney U tests and correlations between variables using Pearson's. P-values <0.05 were considered significant.*

Results. *Shear wave velocity as a measure of skin stiffness was significantly higher in SSc than in controls in 11 out of 16 mRSS sites investigated. Shear-wave velocity was strongly correlated with the local mRSS in the following anatomical sites: forearm, hand, phalanx, and thigh. In the patient group, clinically unaffected skin could also be differentiated from healthy skin using shear-wave velocity.*

Conclusion. *VTIQ represents an innovative and promising technique that provides, for the first time, a non-invasive, absolute quantification of skin stiffness. Further studies of VTIQ are required, but this early study supports the clinical and scientific potential of this new measure of skin involvement in SSc.*

Introduction

Skin involvement is of major clinical and prognostic relevance in systemic sclerosis (SSc) and often the primary outcome in clinical trials (1, 2). Skin thickness is usually measured clinically using the modified Rodnan Skin Score (mRSS), a semi-quantitative measure of cutaneous involvement assessed by palpation (3). However, the mRSS has been criticised for being associated with high inter-observer variability and for requiring a skilled trained investigator (1). There remains therefore, a requirement for an objective and sensitive measure of skin involvement for clinical assessment and especially to support the development of much-needed new interventions.

Elastography is an ultrasound-based imaging modality that, in different forms, has aroused the interest of researchers in ultrasound imaging technology during the last two decades (4). Various approaches to ultrasound elastography have been proposed over the years, including compression-elastography and acoustic radiation force impulse (ARFI) imaging. ARFI imaging is a new method of ultrasound elastography that provides quantitative measurements of tissue stiffness by measuring the velocity of propagation of a shear wave in tissues (5-8). ARFI represents an advance on previous generations of "compression" elastography as the shear wave is generated within the transducer head rather than by the operator and so is less operator-dependent and can be calibrated to produce absolute values for the wave propagation velocity.

A new iteration of ARFI, Virtual Touch Imaging and Quantification (VTIQ) has been proposed to overcome two limitations of previous elastography methods by being more independent of operator variability, and also allowing the use of small adjustable sampling gates in order to assess discrete anatomical structures (9, 10). To-date, this application has been used in the diagnosis of breast, thyroid and liver lesions, par-

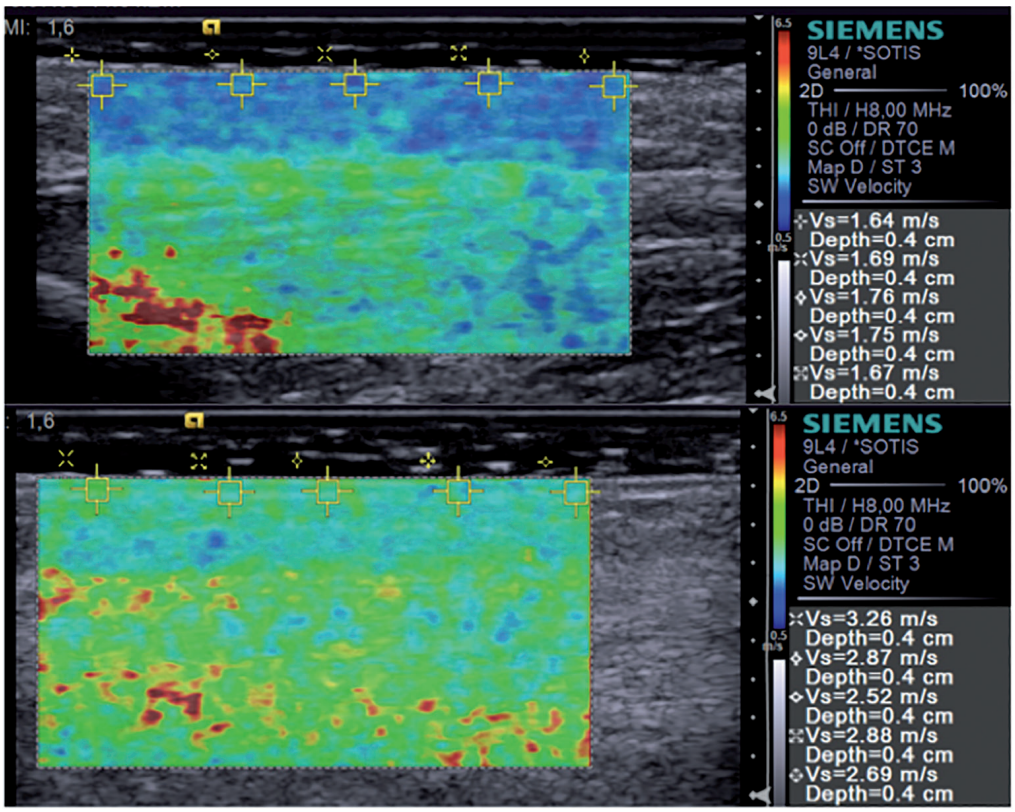

Fig. 1. VTIQ of the dorsal aspect of the hand in a control (**A**) and in a patient with SSc with local skin score = 1 (**B**). Each sampling gate (yellow box) has a corresponding absolute shear-wave velocity (in m/s) on the right side. A higher shear-wave velocity value indicates higher tissue stiffness. E.g. in image A (control), the absolute shear-wave in the first sampling gate in the left is 1.64 m/s. In image B (patient) the first sampling gate in the left is 3.26 m/s.
Tissues with a higher shear wave velocity have higher absolute tissue stiffness. The colour scale depicts graphically the absolute stiffness of all tissues within the region of interest. (red=hard tissue; blue=soft tissue).

ticularly in trying to differentiate benign and malignant tumours (5-8).
We hypothesise that VTIQ may provide a non-invasive sensitive means for absolute quantification of skin stiffness, which may have impact on clinical practice and research in SSc and other fibrosing conditions. The aims of this study were i) to explore the relationship between VTIQ findings and mRSS scores and ii) to use the ability of VTIQ to produce absolute values, to make the first elastographic comparison of skin stiffness in patients with SSc *versus* healthy controls.

Methods

Patients

Twenty-six consecutive patients attending the Scleroderma Outpatient Clinic at our Rheumatology Department (Coimbra, Portugal) participated in the study. All participants fulfilled the American College of Rheumatology/EULAR Classification Criteria for SSc (11). SSc disease was classified into the diffuse and limited subsets according to LeRoy *et al*. (12). Seventeen healthy volunteers were recruited from the hospital staff and patient's family members. The exclusion criteria for the patient group were: having any diagnosis of other skin disorder (*e.g*. psoriasis) and/or overlap with other inflammatory rheumatic diseases.
For the control group the exclusion criteria were: having any diagnosis of other skin disorder (*e.g*. psoriasis) or connective tissue disease or rheumatic inflammatory disease.
Ethical approval was obtained from hospital's ethics committee (Comissão de Ética para a Saúde do Centro Hospitalar e Universitário de Coimbra). All patients and healthy volunteers provided signed, informed consent prior to participation in the study.

Rodnan skin score

Skin involvement in SSc was determined by palpation and semi-quantitative scoring (0-3 ordinal scale: 0= normal skin; 1= slight thickening; 2= moderate thickening; 3= hidebound skin sclerosis) over 17 anatomical sites defined for the mRSS (13). This assessment was performed by a rheumatologist who is fully trained and experienced in this method (MJS), who was blinded to the ultrasound evaluation.

Ultrasound evaluation

The ARFI imaging was performed using an ACUSON S3000™ ultrasound system (Siemens Healthcare) equipped with a linear 4-9MHz transducer. The VTIQ application allows the measurement of shear-wave velocity within the full region of interest and provides size-adjustable sampling gates. A thin layer of gel (approximately 2-5mm) was used to ensure satisfactory contact between the transducer and the skin while allowing visualisation of the epidermis and dermis. The transducer was placed perpendicular to the skin. Acceptance of an ultrasound image for analysis was based on clear visualisation of an interface between the epidermis, dermis and subcutaneous tissues and an automated image quality factor provided by the ultrasound system. The sonographer placed sampling gates with the minimum possible size (2mm x 2mm), over the epidermis and dermis. The VTIQ output simultaneously displays a colour-coded tissue stiffness map and absolute shear-wave velocity measurements (in m/s) in one single image (Fig. 1). A greater shear-wave velocity value indicates higher

Table I. Epidemiological and clinical features of SSc patients and controls.

	SSc patients	Controls
Gender (F/M)	23/3	14/3
Age, mean (SD) (years)	55.3 (12.1)	54.3 (14.8)
Disease duration from RP, mean (SD) (years)	14.9 (9.4)	-
Disease duration from diagnosis, mean (SD) (years)	12.5 (8.7)	-
Disease subset (D/L)	13/13	-
ANA+, n (%)	26 (100.0)	-
ACA+, n (%)	9 (34.6)	-
Antitopoisomerase I+, n (%)	10 (38.5)	-
Anti-PMScl, n (%)	2 (7.7)	-
mRSS, mean (SD)/range	11.8 (9.2)/0–33	-

SSc: systemic sclerosis; F: female; M: male; SD: standard deviation; RP: Raynaud's phenomenon; D: diffuse cutaneous SSc; L: limited cutaneous SSc; ANA: antinuclear antibodies; ACA: anticentromere antibodies; mRSS: modified Rodnan skin score.

Table II. Clinical features and shear-wave velocity values (m/s) in SSc patients and controls.

Rodnan sites	Shear-wave velocity values		Patients *vs.* Controls (*p*-value)	mRSS at the site of analysis, mean (SD)/range	Correlation between mRSS at the site of analysis and SWV, CI 95%
	SSc patients n=26, mean (SD)	Controls n=17, mean (SD)			
Anterior chest	2.7 (1.1)	2.3 (0.7)	NS	0.5 (0.7)/0-2	NS
Abdomen	2.4 (0.8)	2.0 (0.6)	NS	0.2 (0.5)/0-2	NS
Upperarm right	2.5 (1.1)	2.2 (0.5)	NS	0.4 (0.6)/0-2	NS
Upperarm left	2.9 (1.2)	2.3 (0.4)	0.03	0.3 (0.6)/0-2	NS
Forearm right	3.1 (1.2)	2.3 (0.3)	0.005	0.7 (0.7)/0-2	r=0.69, **p<0.01** (0.54,0.85)
Forearm left	2.9 (0.9)	2.1 (0.4)	0.001	1.1 (0.9)/0-3	r=0.68, **p<0.01** (0.5,0.83)
Hand right	4.3 (2.4)	2.2 (1.1)	0.0001	1.1 (0.9)/0-3	NS
Hand left	3.6 (1.8)	2.2 (0.5)	0.001	1.1 (0.9)/0-3	r=0.55, **p<0.05** (0.18, 0,79)
Phalanx right	4.2 (2.2)	2.2 (0.4)	0.0001	1.8 (0.9)/0-3	r=0.67, **p<0.01** (0.52, 0.79)
Phalanx left	4.4 (2.1)	2.3 (0.4)	0.0001	1.7 (1.0)/0-3	r=0.748, **p<0.01** (0.56, 0.88)
Thigh right	2.4 (0.8)	2.1 (0.2)	0.02	0.1 (0.3)/0-1	r=0,543, **p<0.01** (0.34,0.84)
Thigh left	2.4 (0.6)	2.1 (0.3)	0.03	0.1 (0.3)/0-1	r=0,525, **p<0.01** (0.27, 0.81)
Leg right	2.9 (1.2)	2.4 (0.5)	NS	0.4 (0.6)/0-2	NS
Leg left	2.9 (1.3)	2.3 (0.4)	0.04	0.4 (0.6)/0-2	NS
Foot right	3.3 (1.4)	2.2 (0.3)	0.006	0.6 (0.8)/0-3	NS
Foot left	2.3 (0.4)	2.3 (0.4)	NS	0.6 (0.8)/0-3	NS

NS: Not statistically significant.

tissue stiffness. The shear wave velocities within discrete sampling gates are displayed instantaneously in a quantitative data box, at the right side of the image. The mean value for each site scanned was obtained from three measurements per site. The probe was lifted off and replaced in the same region as in the previous measurement.

The scanned skin regions included both clinically involved (mRSS=1, 2, 3) and uninvolved (mRSS=0) skin. Every region assessed for the mRSS was scanned. To ensure the maximum reproducibility between measurements we carefully replicated the anatomical sites and landmarks recommended for the Rodnan skin score (14). The mRSS value reflects the worst score in a region. The elastography probe was performed in that specific spot. The shear-wave velocity (SWV) measurements were performed three times at the same location. The three consecutive SWV measurements were used to calculate the mean for the statistical analysis.

Four SSc patients and two controls underwent repeat ultrasound evaluation in two different scanning sessions, one week apart, by the same operator. The mRSS at the sites of analysis used in the reliability sub-study ranged from 0 to 3.

Statistical analysis

Data were analysed using SPSS software, V.21 (IBM SPSS Inc., Chicago, IL, USA) with *p*-values <0.05 being considered significant. Continuous variables were reported as means ±1standard deviation, if normally distributed; or median plus interquartile range, if not normally distributed. Categorical variables were presented as frequencies. Comparison between groups was performed using the Mann-Whitney U test. Correlations between ultrasound measurements and total and site–specific mRSS scores were calculated using the Pearson's correlation test. The intraclass correlation coefficient was calculated to examine the intra-observer reliability of ultrasound measurements.

Results

The demographic and clinical characteristics of the 26 SSc patients and 17 healthy controls are presented in Table I. Shear wave velocities measurements were significantly higher in SSc patients than in controls in 11 out of 16 mRSS sites of analysis (Table II). Sites where the differences did not reach statistical significance were those that were also frequently unaffected at clinical examination.

The skin stiffness was highly correlated with the local mRSS in the following anatomical sites: forearm right and left, hand left, phalanx right and left and thigh right and left (Table II).

In order to compare absolute skin stiffness values in regions of clinically unaffected scleroderma skin (mRSS=0) with the skin of healthy controls, we compared shear wave velocities at these sites with those obtained in similar skin regions of healthy controls. Absolute skin stiffness measurements were higher in all SSc "unaffected" areas than in the respective site of the healthy controls, reaching statistical significance in eight out of 16 measurement sites (Table III).

Table III. Shear wave velocities values (in m/s) in unaffected skin sites from SSc patients and controls.

	Patients [*]	Controls	*p*-value
Anterior chest	2.8 (0.7) [17]	2.3 (0.7)	0.05
Abdomen	2.5 (0.4) [21]	2.0 (0.6)	0.016
Upperarm left	2.7 (0.5) [19]	2.3 (0.4)	0.004
Upperarm right	2.4 (0.4) [18]	2.2 (0.5)	NS
Forearm left	2.5 (0.3) [14]	2.1 (0.4)	0.008
Forearm right	2.5 (0.4) [13]	2.3 (0.3)	NS
Hand left	2.6 (0.4) [7]	2.2 (0.5)	NS
Hand right	2.7 (0.4) [7]	2.2 (1.1)	0.011
Phalanx left	2.7 (0.4) [1]	2.3 (0,4)	-
Phalanx right	3.1 (0.8) [3]	2.2 (0.4)	-
Thigh left	2.3 (0.6) [23]	2.1 (0.3)	NS
Thigh right	2.4 (0.4) [23]	2,1 (0.2)	0.004
Leg left	3.1 (1.0) [19]	2.4 (0.4)	0.012
Leg right	2.6 (0.5) [18]	2.5 (0.5)	NS
Foot right	2.9 (0.7) [14]	2.2 (0.3)	0.003
Foot left	2.6 (0.6) [15]	2.3 (0.4)	NS

[*] Number of patients with mRSS =0 at each site. Shear wave velocities values are shown as mean (standard deviation). $p<0.05$ was considered statistically significant. NS: Not statistically significant.

Table IV. The Intra-observer variability between shear wave velocity (SWV) measurements of subjects at the baseline and one week later.

Site	ICC	*p*-value
Anterior chest	0.478	0.246
Abdomen	0.794	0.054
Upperarm left	0.839	0.033
Upperarm right	0.828	0.038
Forearm left	0.920	0.007
Forearm right	0.919	0.008
Hand left	0.720	0.122
Hand right	0.787	0.057
Phalanx left	0.984	0.001
Phalanx right	0.981	0.001
Thigh left	0.769	0.067
Thigh right	0.740	0.083
Leg left	0.827	0.038
Leg right	0.922	0.007
Foot right	0.833	0.036
Foot left	0.804	0.049

ICC: intraclass correlation coefficient.

The intraobserver reliability was explored in a small group of patients, and shown to be excellent for forearm and phalanx (ICC >0.9, suggesting that VTIQ quantification could be a reliable tool in assessment of skin stiffness (table 4). We did not assess the intra-observer variability of mRSS but this has been shown to be in the range of 10-15% (15).

Discussion

This is the first study reporting the application of VTIQ method for the absolute quantification of skin stiffness in SSc. Although a small number of studies have reported manual compression elastography in SSc, they have provided only qualitative images and stiffness values that are relative to other tissues within the region of interest (16, 17).

Absolute skin stiffness values in 11 out of 16 sites scanned were significantly higher in SSc patients than in healthy controls, suggesting that VTIQ may have the potential to be a valuable tool in quantitative assessment of skin involvement in SSc. A strong correlation between VTIQ and local mRSS was observed in only four anatomical areas - forearm, hand, phalanx and tight. The lower correlations in other sites will be at least in part due to the low range of scores and lower mRSS scores reported in those locations. This suggests that VTIQ may in future also have a role in differentiating subclinical skin changes, ie in sites with a clinical mRSS score of zero. Table II shows considerable differences in shear wave velocity between the right/left sides of some anatomical sites in SSC patients. This might suggest that the skin involvement in SSc may be less symmetrical than clinically expected, and highlights the discriminant ability of VTIQ in detecting subtle skin changes. We acknowledge that VTIQ and skin score do not measure exactly the same properties of the skin. The mRSS measures not only thickness, but also texture and fixation (18, 19), while VTIQ measures only the stiffness of the skin. These differences must also be taken into account when considering correlations between the two methods in this study and future applications of VTIQ.

Our results indicate that VTIQ is capable of identifying significantly increased stiffness in skin from SSc sites considered clinically 'unaffected'. This is in agreement with recent microarray gene expression studies suggesting that clinically unaffected skin shares the peculiar gene signatures and the pathology of clinically affected skin in SSc (20-22). The increased sensitivity of VTIQ in the early stages of skin involvement may represent a valuable potential contribution to clinical assessment, and especially to research into the pathogenesis and treatment of this disease.

Our results need to be evaluated in the light of some study limitations. The number of patients assessed is relatively small although the new data indicate that further formal studies in larger populations are warranted. The reference values and the distribution of measured SWV in healthy controls have not yet been defined. Our cross-sectional design also does not allow conclusions or inferences regarding the technique's sensitivity to change over time or in response to treatment. The intraobserver reliability was explored only superficially, although it was excellent (ICC>0.9) for forearm and phalanx, suggesting that VTIQ quantification could be a reliable tool in assessment of skin stiffness. Interobserver reliability, a key feature of any evaluation method, was not addressed in this study. Operator reliability is person/equipment and cohort specific and will need further evaluation prior to using the VTIQ technique in larger studies.

Clinically, VTIQ has no significant contraindications and allows the examination of multiple sites and tissue types in a single session. Furthermore, it is a relatively user-friendly technique that requires minimal operator training. The technique takes less than two minutes per site examined and offers the advantage of being able to save image files for further operator-independ-

ent analysis. The total duration of the VTIQ assessment will depend on the number of anatomical sites examined, which may actually be reduced in the future if the higher precision and reliability of VTIQ are confirmed. The time consumption may, in fact, be compensated by several factors, including the improved precision, reproducibility and quantification of the measurements. As well as, the ability to save image files for further operator-independent analysis. VTIQ offers an increased range of quantification for skin assessment in SSc patients compared with semi-quantitative mRSS; the latter limited to 4 integer values, which restricts the ability of the mRSS to record subtle improvement or worsening that may still be clinically important. In addition, this technique represents an advance on previous generations of "manual compression" elastography as the input wave is produced by the transducer rather than by the operator, thus minimising operator effects, and an automated image quality factor is provided to allow clinicians to select the highest quality image.

Our experience with this technique suggests that there remains room for technical improvement: 1. Although the sampling gate can be reduced to 2mmx2mm, this is still too large for certain anatomical structures (eg, epidermis and dermis); VTIQ is also currently only available with a low frequency ultrasound probe (9 MHz), which limits the resolution at which cutaneous and sub-cutaneous structures can be imaged. Other imaging techniques such as optical coherence tomography will likely retain primacy for imaging the most superficial epidermal structures (23).

In conclusion, our findings indicate that VTIQ represents meaningful progress in the quantitative assessment of skin stiffness. VTIQ has the potential to add greater precision and objectivity to the non-invasive assessment of skin involvement in SSc, which may be important in clinical care and in the evaluation of new interventions. Longitudinal studies in larger samples are now required to assess inter-observer reliability and sensitivity to change over time.

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