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Normality reference of ultrasound-dermal thickness in healthy controls

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Normality reference of ultrasound-dermal thickness in healthy controls

Artigo Científico Original

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LIST OF ABBREVIATIONS

BMI - Body mass index

CI - Confidence intervals

EUSTAR - European Scleroderma Trials and Research group

HFUS - High-frequency ultrasound

IQR - Interquartile range

MCF - Metacarpophalangeal

MTF – Metatarsophalangeal

mRSS - Modified Rodnan skin score

SD - Standard deviation

SSc - Systemic sclerosis

ABSTRACT

Introduction: Increase evidence supports the application of high-frequency ultrasound (HFUS), as a diagnostic and monitoring tool for skin assessment in clinical practice and research, in systemic sclerosis (SSc). However, knowledge is lacking on the factors that influence ultrasound-dermal thickness in normal individuals, which hampers the ability of HFUS to contribute to the early diagnosis of SSc and the interpretation of skin involvement in patients with established disease. Factors such as age and gender may deserve consideration when interpreting skin ultrasound measures and their application in SSc.

Objective: To determine normal reference values of ultrasound-dermal thickness, in Rodnan skin sites, taking in account the impact of age and gender on these measures.

Methods: A cross-sectional study was conducted among normal individuals aged 20–79 years. Recruitment was stratified by gender and age (10-year categories). Ultrasound-dermal thickness was assessed by HFUS at the 17 skin sites of the modified Rodnan skin score (mRSS). Descriptive statistics were used to describe the overall distribution of ultrasound-dermal thickness measures across skin sites, age and gender categories. The association between age, gender and ultrasound-dermal thickness measures for each of the skin sites was performed through univariable and multivariable linear regressions.

Results: 140 volunteers were included. A gender impact was found in ultrasound-dermal thickness for all Rodnan skin sites (except in the chest). In addition, age was associated with ultrasound-dermal thickness affecting differently males and females. In females, age was a significant predictor at the face and leg, whereas in males, this was observed in the face, forearm and hand.

Conclusion: Normal reference values of ultrasound-dermal thickness were described for each Rodnan skin site and may serve as cut-off levels for ‘normal’ versus ‘abnormal’, thus supporting an earlier diagnosis of SSc.

Keywords: dermal thickness, high-frequency ultrasound, normality reference, skin, systemic sclerosis.

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune rheumatic disease with high clinical burden and unmet needs which is predominantly due to vascular damage and fibrosis of the skin and/or internal organs.[1] Skin fibrosis is a hallmark of the disease [2]. The natural history of SSc is complex and skin involvement comprises three distinct phases: early inflammatory, fibrotic/indurative and atrophic phase.[3] However, the judgment of skin thickness is often difficult to distinguish clinically.[3] Skin involvement is an important marker of disease activity [4], severity and prognosis [2] making its assessment a key issue in clinical practice and research.[5] In addition, the extent of skin involvement and its rate of progression are associated with survival, internal organ involvement [6] and functional disability.[7] Modified Rodnan skin score (mRSS), the current gold standard to evaluate skin involvement, is a semi-quantitative score based on the palpation of the skin on 17 anatomical sites.[8] It is often used as primary or secondary outcome in clinical trials.[9,10] The mRSS is also a major component of the composite response index in diffuse cutaneous SSc[11] and it is included in the EUSTAR disease activity score.[12] However, mRSS has several limitations, including interobserver variability,[13] high level of subjectivity,[14] inability to discriminate phases of scleroderma skin[15] and low sensibility to skin changes.[16] Thus, it is easily recognized the importance of a correct diagnosis and evaluation of the degree of skin involvement for the successful clinical management of this disease.[17]

Over the last three decades, increasing evidence supports the application of skin high-frequency ultrasound (HFUS) as a diagnostic and monitoring tool in clinical practice and research. [5,16,18,19] This has been possible through technological improvements, namely due to higher frequency transducers.[20,21] Advantages of skin HFUS over the mRSS include, in particular, its objectivity, higher intra- and inter-reproducibility and sensitivity to detect minimal changes, which can definitely contribute to improve the management of patients with SSc.[16,22] Interestingly, HFUS studies in SSc have found skin changes in areas considered unaffected by physical examination. [14,23] Sulli et al. found that patients with limited SSc had ultrasound-dermal thickness higher than healthy subjects, including in sites defined as clinically unaffected (ie, mRSS local =0).[23] Other studies also suggest that HFUS may be able to identify the oedematous phase, in early stages of the disease.[13,16]

Thus, skin HFUS is a promising diagnostic and monitoring tool in clinical practice and research of SSc patients. [5,16] However, there is a lack of sound evidence concerning the factors affecting skin ultrasound measures among normal individuals. Imperative factors such as gender, age and skin site are not often taken in consideration when interpreting skin ultrasound measures.[23] Performing measurements on healthy controls may provide a baseline of comparison and represent an important step to improve interpretation of these measures in SSc patients - not

only to perform an early diagnosis, but also to monitor disease evolution over time or treatment response, in a non-invasive way.[5] Ultimately, normality reference values may be of importance to establish cut-off levels for ‘normal’ versus ‘abnormal’ [5], objectively determining when do the patient starts deviating from his/her ‘personal normality values’.

Therefore, research with focus on developing a unified and general understanding of ultrasound-dermal thickness measured by HFUS is urgent to sustain its utility as a reliable and feasible tool in SSc, particularly to assess patients with an undifferentiated connective tissue disease at risk for SSc, early SSc diagnosis or a SSc patient with non-apparent clinical skin involvement.

The aim of the present study was to determine normal reference values of ultrasound-dermal thickness, in Rodnan skin sites, among 140 normal individuals, taking in account the impact of age and gender on these measures.

METHODS

Study population

A cross-sectional study was conducted, aiming at including 140 participants aged 20-79 years. Participants were recruited among the hospital staff, patients' family members and University students.

Recruitment was stratified for age (10-year categories) and gender, intending to guarantee a balanced distribution of these factors in the population. Considering that the age of onset of SSc is most commonly in the range of 30–50 years [17], we have added 10 more participants in these groups, in females. The sample size of approximately 140 participants is based mainly on convenience, and the requirement that we would like to have at least 10 participants per stratum.

All participants met the following exclusion criteria: 1) pregnancy; 2) having diagnosis of any skin disease, connective tissue disease or rheumatic inflammatory disease; 3) past history of treatment with cancer chemotherapy; 4) history of exposure to organic solvents; 5) current or recent (<4 weeks) treatment with glucocorticoid, and 6) past history of glucocorticoid treatment for more than four months, regardless of clinical indication.

The following parameters were recorded at the time of the study: age, gender, body mass index, menopause status, smoking habits and medication (current and past).

Ethical approval was obtained from the Ethics Committee of Centro Hospitalar e Universitário de Coimbra (CHUC – 118-17). All methods and procedures were explained to each participant, and all of them provided signed informed consent.

Ultrasound-dermal thickness

All measures were performed before noon (between 8:30 and 12:30) in the same room at a temperature between 21º and 23ºC, and after an acclimatization period of 15 minutes, with the patient lying in a supine and relaxed position. Each set of measurements took approximately 20 minutes.

Ultrasound evaluation was performed at the 17 sites of the mRSS, as follows: face, chest (between sternal angle and notch), upper arm (anterior aspect, 10 cm proximal to the medial epicondyle), forearm (anterior aspect, 3 cm proximal of the wrist), hand dorsum (index/middle

metacarpal interspace, 2cm proximal to the MCF joints), finger (dorsal aspect of the mid portion of the proximal phalanx of the right second finger), abdomen (10 cm distal to the sternum); thigh (10 cm proximal to the patella), leg (10 cm proximal to the lateral malleolus), and foot (first web space 2 cm proximal to the MTF joints).[13] All Rodnan skin sites were evaluated bilaterally, with exception to face, chest and abdomen.

Skin ultrasound was performed using a Siemens ACUSON S2000 Ultrasound System HELX Evolution.

B-mode ultrasound was performed using an 18 MHz linear probe. A high-frequency probe offers considerably good resolution, allowing the distinction between the epidermis, dermis and subcutaneous layers of skin.[13] In particular, ultrasound-dermal thickness was measured on the B-mode image by an electronic caliper included in a dedicated software, identifying the upper surface epidermis-dermis and the lower layer dermis-subcutis. The value for each skin site scanned was calculated as the mean of three measurements per site, in millimetres (mm).

The same operator (TS) performed the US evaluations in all individuals, blinded to the mRSS, and recorded the relevant scans. Then, four operators (Tânia Santiago, João Lima, Catarina Gaspar and Mariana Luís) read the ultrasound scans, using an exact standardization protocol and specific software (Dicom viewer).

Statistical analysis

Descriptive statistics (mean, median, standard deviation, confidence interval 95%, inter-quartile range, as appropriate) were used to describe the overall distribution of ultrasound-dermal thickness measures across skin sites, age and gender categories. Comparisons of ultrasound measures between dominant and non-dominant sides were performed using paired-samples T test or Wilcoxon matched-pair signed rank test, as appropriate (excluding the left-handed controls) (data not shown).

The association between age and gender and ultrasound-dermal thickness measures for each of the skin sites was performed through linear regressions. Univariable followed by multivariable linear analysis were performed to identify factors influencing ultrasound measures. The analyses were performed separately for all the controls (n=140) and for males (n=60) versus females (n=80).

Data were analyzed using SPSS software with p values < 0.05 being considered significant.

RESULTS

A total of 140 participants were included in this study. All the age and gender categories were complete. The participants' features are presented in Table 1.

TABLE 1 | Demographic characterization of the study participants.

Covariate	Frequency
Gender, %	
Male	43
Female	57
Age categories, n	
20-29	20
30-39	30
40-49	30
50-59	20
60-69	20
70-79	20
BMI (Kg/m²), %	
BMI <25	44
BMI >25	56
Smoking, %	
Never	70
Past	17
Current	13
Menopause status, %	
Yes	50
No	50

The values of ultrasound-dermal thickness measured by high-frequency ultrasound, at all Rodnan skin sites, are presented in Table 2. The ultrasound-dermal thickness was highest in the abdomen (1.67 ± 0.37), and lowest in the fingers (0.62 ± 0.10). We haven´t found any significant difference between the left and right skin sites. Thus, the values of ultrasound-dermal thickness are presented as the mean for both sites.

TABLE 2 | Values of ultrasound-dermal thickness (in mm) measured by high-frequency ultrasound, at Rodnan skin sites, in normal individuals (aged 20-79 years, males and females).

Rodnan skin sites	Mean (SD)	95% CI lower,upper	Minimum-Maximum	Median	IQR
Face	1.26 (0.33)	1.19 to 1.32	0.42 - 2.53	1.22	0.36
Chest	1.41 (0.38)	1.34 to 1.48	0.53 – 2.67	1.33	0.42
Abdomen	1.67 (0.37)	1.59 to 1.74	0.81 – 2.81	1.66	0.47
Upperarm	0.81 (0.15)	0.78 to 0.83	0.45 – 1.19	0.82	0.18
Forearm	0.83 (0.19)	0.79 to 0.87	0.47 – 1.43	0.81	0.25
Hand	0.64 (0.12)	0.62 to 0.67	0.40 – 1.02	0.62	0.18
Finger	0.62 (0.10)	0.59 to 0.64	0.43 – 0.89	0.61	0.16
Thigh	1.36 (0.31)	1.30 to1.41	0.56 – 2.44	1.33	0.44
Leg	0.95 (0.33)	0.89 to 1.01	0.42 – 1.98	0.97	0.49
Foot	0.71 (0.16)	0.68 to 0.73	0.44 – 1.38	0.70	0.20

SD, standard deviation; CI, confidence intervals; IQR, interquartile range.

TABLE 3 | Mean and 95% confidence interval of ultrasound-dermal thickness measures across age categories, in males (n=60) and females (n=80).

Rodnan skin sites	Gender	20-29y	30-39y	40-49y	50-59y	60-69y	70-79y
Face	Males	1.93 (1.29 to 2.57)	1.79 (1.32 to 2.27)	1.45 (1.23 to 1.66)	1.29 (1.04 to 1.53)	1.47 (1.23 to 1.72)	1.20 (0.83 to 1.58)
	Females	1.19 (1.07 to 1.31)	1.25 (1.18 to 1.32)	1.16 (1.08 to 1.23)	1.10 (0.96 to 1.25)	1.06 (0.91 to 1.20)	0.89 (0.68 to 1.10)
Chest	Males	1.44 (1.06 to 1.83)	1.51 (1.16 to 1.86)	1.38 (1.16 to 1.61)	1.29 (1.01 to 1.56)	1.35 (1.09 to 1.61)	1.44 (1.15 to 1.73)
	Females	1.14 (1.03 to 1.25)	1.49 (1.29 to 1.68)	1.55 (1.33 to 1.76)	1.57 (1.26 to 1.88)	1.26 (1.03 to 1.49)	1.38 (0.83 to 1.94)
Abdomen	Males	1.65 (1.34 to 1.96)	1.87 (1.55 to 2.19)	1.88 (1.61 to 2.15)	1.79 (1.56 to 2.03)	1.91 (1.54 to 2.28)	1.73 (1.39 to 2.06)
	Females	1.56 (1.36 to 1.76)	1.66 (1.47 to 1.84)	1.59 (1.39 to 1.80)	1.62 (1.47 to 1.77)	1.53 (1.19 to 1.87)	1.40 (1.11 to 1.69)
UpperArm	Males	0.93 (0.62 to 1.24)	0.93 (0.82 to 1.03)	0.87 (0.76 to 0.97)	0.91 (0.79 to 1.02)	0.91 (0.79 to 1.03)	0.84 (0.73 to 0.95)
	Females	0.71 (0.60 to 0.81)	0.78 (0.73 to 0.84)	0.75 (0.69 to 0.80)	0.75 (0.69 to 0.80)	0.69 (0.59 to 0.81)	0.65 (0.49 to 0.79)
Forearm	Males	0.99 (0.89 to 1.09)	1.02 (0.81 to 1.23)	1.02 (0.89 to 1.14)	0.99 (0.75 to 1.24)	0.87 (0.73 to 1.01)	0.73 (0.63 to 0.82)
	Females	0.69 (0.60 to 0.78)	0.84 (0.77 to 0.89)	0.84 (0.76 to 0.92)	0.71 (0.62 to 0.79)	0.75 (0.61 to 0.88)	0.71 (0.49 to 0.92)
Hand	Males	0.85 (0.76 to 0.94)	0.81 (0.69 to 0.91)	0.71 (0.59 to 0.82)	0.70 (0.62 to 0.79)	0.71 (0.66 to 0.76)	0.58 (0.49 to 0.66)
	Females	0.59 (0.55 to 0.63)	0.63 (0.59 to 0.67)	0.65 (0.58 to 0.72)	0.55 (0.49 to 0.61)	0.53 (0.47 to 0.59)	0.58 (0.50 to 0.66)
Finger	Males	0.72 (0.27 to 1.17)	0.72 (0.65 to 0.79)	0.68 (0.59 to 0.77)	0.68 (0.56 to 0.79)	0.69 (0.63 to 0.74)	0.51 (0.46 to 0.57)
	Females	0.55 (0.52 to 0.58)	0.61 (0.57 to 0.65)	0.63 (0.58 to 0.68)	0.56 (0.52 to 0.61)	0.56 (0.51 to 0.62)	0.55 (0.45 to 0.65)
Thigh	Males	1.53 (1.38 to 1.67)	1.55 (1.25 to 1.86)	1.57 (1.33 to 1.82)	1.33 (1.14 to 1.51)	1.61 (1.19 to 2.03)	1.48 (1.24 to 1.72)
	Females	1.22 (1.05 to 1.39)	1.25 (1.13 to 1.36)	1.19 (1.08 to 1.30)	1.18 (0.98 to 1.38)	1.39 (1.26 to 1.51)	1.43 (1.14 to 1.72)
Leg	Males	1.17 (0.98 to 1.36)	1.09 (0.75 to 1.44)	1.24 (0.94 to 1.54)	1.00 (0.89 to 1.11)	1.01 (0.70 to 1.33)	1.15 (0.66 to 1.64)
	Females	0.93 (0.77 to 1.08)	1.01 (0.90 to 1.12)	0.99 (0.89 to 1.09)	0.59 (0.48 to 0.70)	0.69 (0.47 to 0.90)	0.75 (0.54 to 0.95)
Foot	Males	0.80 (0.69 to 0.92)	0.85 (0.71 to 0.99)	0.76 (0.62 to 0.89)	0.81 (0.66 to 0.96)	0.74 (0.64 to 0.85)	0.79 (0.51 to 1.07)
	Females	0.63 (0.57 to 0.69)	0.66 (0.62 to 0.71)	0.71 (0.66 to 0.76)	0.59 (0.50 to 0.68)	0.69 (0.57 to 0.81)	0.65 (0.48 to 0.81)

Values are expressed in millimetres.

Table 4 presents the impact of age and gender on ultrasound-dermal thickness, in all skin sites.

Multivariate linear regression (table 4 and table S1) showed that gender in general had impact on dermal thickness in all skin sites, particularly in the face (β = -0.533, p<0.0001), upperarm (β = -0.486, p<0.0001) and hand (β = -0.448, p<0.0001).

Age was particularly associated with ultrasound-dermal thickness in the face (β = -0.368, p<0.0001), leg (β = -0.264, p=0.001), forearm (β = -0.258, p=0.001) and hand (β = -0.248, p=0.001).

TABLE 4 | Impact of age and gender on all participants (n=140) on ultrasound-dermal thickness, in the multivariate linear regression.

	Face	Chest	Abdomen	UpperArm	Forearm	Hand	Finger	Thigh	Leg	Foot
AGE	+	✕	✕	+	+	+	✕	✕	+	✕
GENDER	++	✕	+	++	++	++	+	+	++	+

✕ no significative impact of the variable
++ higher impact of the variable (higher β standardized values) (please see Appendix III – Table S1)
+ moderate impact of the variable (lower β standardized values) (please see Appendix III – Table S1)

For both genders (table 5 and table S1), ultrasound-dermal thickness of the face decreases significantly with advancing age (males: β= -0.428, p=0.001 and females: β = -0.473, p<0.0001). In addition, age in males was a stronger predictor of ultrasound-dermal thickness in the hand (β = -0.374, p=0.003) and forearm (β = -0.479, p<0.0001) than in females.

TABLE 5 | Impact of age on FEMALES (n=80) and MALES (n=60) on ultrasound-dermal thickness, in the univariable linear regression.

	Face	Chest	Abdomen	UpperArm	Forearm	Hand	Finger	Thigh	Leg	Foot
FEMALES	+	✕	✕	✕	✕	✕	✕	✕	+	✕
MALES	+	✕	✕	✕	+	+	✕	✕	✕	✕

✕ no significative impact of the variable
+ significant impact of the variable

DISCUSSION

In the present study, gender (and, in some skin sites, age) had a significant impact on ultrasound-dermal thickness measured at Rodnan skin sites. This is, to our knowledge, the first study investigating the association of age and gender with skin ultrasound measures performed in a systematic approach and including all 17 Rodnan skin sites.[13]

An important gender impact was found in ultrasound-dermal thickness for all Rodnan skin sites (except in the chest). In all the Rodnan skin sites evaluated, males had significantly higher ultrasound-dermal thickness values than females. Our findings are in line with previous ultrasound studies. One study including 30 healthy subjects (17 females, 13 males) have also found that male ultrasound-dermal thickness was significant higher than females, in two (neck and dorsum of foot) out of 5 skin sites evaluated (cheek, neck, hand palm, foot dorsum and sole), using a 22 MHz probe.[24] In another study, Seidenari et al. included 48 controls (24 females and 24 males) and described that skin thickness was significant higher in males, at 4 skin sites evaluated (forehead, cheek, back and lower forearm), using a 20 MHz probe.[25]

Secondly, we found an important association between age and ultrasound-dermal thickness affecting differently males and females. Particularly, in females, age was a significant predictor of skin thickness at the face and leg, while in males this was observed in the face, forearm and hand. In fact, histological data indicates that in elderly skin, the amount of collagen in the dermis decreases and degeneration of the elastin network occurs which may alter density, composition and structure of dermal layer.[26,27] Previous biopsy-based studies found a decrease of dermal thickness with increasing age [28,29,30,31], although this pattern of decreasing may differ among males and females.[28] Some studies even outlined a tendency of skin thickness to increase in the 21 to 40 year period and decrease after 60-70 years of age.[32]. A biopsy study of the dorsal surface of the forearm had shown that in males skin thinned gradually with age, while in females thickness was constant until age of 40, decreasing thereafter.[28] Also, Branchet et al. found a decrease in dermal thickness in the upperarm, particularly between 20-30 and 70-80 years for males and for females.[29]

In addition, previous published skin ultrasound studies have in general corroborated our findings. A study using a 25 MHz probe, A mode, investigating the impact of age on skin in 54 males and 69 females, reported a decrease on skin thickness of ventral forearm after age 70. Moreover, it also showed that male skin was thicker than that of females throughout the age range (0-90+).[33] Similarly, Kozarova A. et al. concluded that skin thickness (epidermis plus dermis) of the dorsal forearm and ventral thigh significantly thinner in subjects older than 65 years.[34] Lastly, another ultrasound study focusing on volar and dorsal aspect of the forearm of

142 females (age ranging between 0-10 up to 80-90 years) found significant thinning of the dermis only after eighth and seventh decade on volar and dorsal forearm, respectively.[35]

Comparison between our results and from previous ultrasound studies in normal individuals is hampered by several factors, such as different skin sites assessed (or even if the same skin sites were assessed, often different landmarks were used) and ultrasound technique (eg, equipment and transducer frequency). In fact, ultrasound studies have underscored the importance of standardized conditions when performing ultrasound skin. Furthermore, our study seems to be unique in the recruitment stratified for age and gender, in order to obtain a sample in which these factors are balanced.[23,25,27,31]

Some limitations of the present study should be addressed. The sample size is relatively small, and it is not certain to what extent these results are generalizable to other populations. We accepted any volunteer, but the eligibility criteria - which focused on factors eventually affecting skin properties - were strictly assessed to ensure we would have a population composed of individuals with normal skin assessment. Factors potentially influencing skin ultrasound measures such as body mass index, menopause status and smoking habits, but this was not taken in consideration for the present study. In future studies, such factors should be taken in consideration to investigate its impact on ultrasound-thickness measures and to reach consensus about normality reference values weighting these factors. Lastly, a linear transducer 18MHz was used to evaluate ultrasound-dermal thickness which may limit the image resolution, although this frequency is the most commonly used in skin ultrasound studies and frequently available in clinical practice.

Notwithstanding these limitations, this study presents strengths, such as the solid standardized protocol, having an homogeneous sample in terms of factors influencing skin ultrasound measures and a population of normal individuals, evaluating only dermis thickness (instead of total skin thickness), and also including a comprehensive evaluation with all the 17 Rodnan skin sites.

In summary, a gender (and, in some skin sites, age) association was found in ultrasound-dermal thickness in Rodnan skin sites, i.e. dermal thickness decreased overall with age and was higher in males than in females. A normality reference range for each Rodnan skin site was described, but we believe it requires validation with future ultrasound studies in other populations. Nevertheless, our study may guide as a benchmark in research and clinical practice settings when assessing the skin of patients with SSc. In practice, it may be of value to assess a patient with an Undifferentiated Connective Tissue Disease at risk for SSc or with

non-apparent clinical skin involvement rigorously when he starts deviating from his ‘personal curve’. This hypothesis needs to be further investigated, but it looks to be a promising use of normality reference values**.**

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FINAL NOTES

My role in this project was to read and evaluate the skin ultrasound images and write this manuscript, under the orientation of MD Tânia Santiago and PHD, MD José António Pereira da Silva. I and my colleague, João Lima, have had training sessions to read and evaluate the skin ultrasound images (~3hours total), led by MD Tânia Santiago.

I have co-authored and collaborated in one Abstract submitted to 2021 European League Against Rheumatism congress entitled: *Ultrasound assessment of dermal thickness and stiffness in undifferentiated connective tissue at risk for systemic sclerosis* (Please See Appendix I and II).

APPENDICES

Appendix I: Submission to 2021 EULAR congress notification

Abstract submission notification

Dear Catarina Gaspar,

Thank you for your submitting your abstract to **EULAR 2021 Congress.**

Details of your abstract

Number:	3384
Title:	Ultrasound assessment of dermal thickness and stiffness in undifferentiated connective tissue disease at risk for systemic sclerosis
Presenting Author:	MD Tânia Santiago
Authors: Tânia Santiago	Centro Hospitalar e Universitário de Coimbra; Centro Hospitalar e Universitário de Coimbra; Rheumatology
Authors: Mariana Luis	Centro Hospitalar e Universitário de Coimbra; Centro Hospitalar Universitário de Coimbra; Rheumatology
Authors: João Lima	Institute for Clinical and Biomedical Research (iCBR)
Authors: Catarina Gaspar	xx
Authors: Maria Joao Salvador	Centro Hospitalar e Universitário de Coimbra
Authors: José Antonio P. da Silva	Centro Hospitalar e Universitário de Coimbra; Centro Hospitalar e Universitário de Coimbra; Rheumatology

Appendix II: Abstract Submitted to 2021 EULAR congress

Ultrasound assessment of dermal thickness and stiffness in undifferentiated connective tissue disease at risk for systemic sclerosis

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2- Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

Background: High-frequency ultrasound (HFUS) and shear-wave elastography (SWE) allow an objective assessment of skin involvement in systemic sclerosis (SSc) patients.[1] Till now it has been applied to patients with established diagnosis.[2,3] However, there is no data concerning its application in Undifferentiated Connective Tissue Disease at risk for SSc (UCTD-risk-SSc), i.e., patients with Raynaud's phenomenon and either SSc marker autoantibodies or typical capillaroscopic findings or both, not satisfying classification criteria for SSc.[4] Our aim was to compare ultrasound-dermal thickness (DT) and skin stiffness using high-frequency ultrasound and shear-wave elastography, in UCTD-risk-SSc and healthy controls.

Methods: Forty UCTD-risk-SSc patients and 40 age- and gender-matched healthy controls were included. Ultrasound-DT was measured using a 18MHz probe, and skin stiffness (i.e. shear-wave velocity values, SWV) using the VTIQ software with a 9MHz probe, at the 17 Rodnan skin sites. Continuous data were expressed as the mean (SD), and Mann-Whitney U test was performed to compare differences between the groups as variables were not normally distributed. Associations between variables were analyzed using the Spearman's correlation.

Results: SWV values were significantly higher in patients with UCTD-risk-SSc compared with controls at the right and left hands, and in the right and left fingers (table 1). Higher values of ultrasound dermal-thickness were found in the fingers and hands bilaterally, although differences were only significantly at the hands, compared with healthy controls (table 1). There were no significant differences in the other Rodnan skin sites. There was no significant correlation between ultrasound-dermal thickness and stiffness at the same skin site.

Conclusions: This study provides the first evidence suggesting that ultrasound-DT and stiffness can discriminate patients with UCTD-risk-SSc from healthy controls. Prospective studies including a larger number of patients with different subsets of UCTD-risk-SSc are needed to investigate diagnostic and prognostic value of the ultrasound parameters in this group.

Table 1. Clinical and ultrasound parameters in UCTD-risk-SSc and healthy control groups.

	UCTD-risk-SSc (n=40)	**Healthy controls (n=40)**	**p value**
Age, mean (SD)	51.4 (14.9)	49.8 (13.9)	Ns
Female, n (%)	36	36	
Raynaud phenomenon, %	100.0%	-	
ANAs Anti-centromere, % Anti-Scl70+, %	100.0 60.0 11.5	-	
Scleroderma/non-scleroderma pattern in capillaroscopy, %	5.0/95.0	-	-
Ultrasound parameters			
Dermal thickness (mm)			
Dorsal hand Right	0.77 (0.32)	0.62 (0.12)	0.02
Dorsal hand Left	0.79 (0.39)	0.62 (0.13)	0.02
Proximal phalanx right	0.64 (0.14)	0.61 (0.11)	Ns
Proximal phalanx left	0.66 (0.16)	0.60 (0.09)	Ns
SWV values (m/s)			
Dorsal hand Right	1.94 (0.40)	1.61 (0.24)	0.0001
Dorsal hand Left	1.82 (0.36)	1.65 (0.25)	0.025
Proximal phalanx right	2.09 (0.60)	1.68 (0.24)	0.001
Proximal phalanx left	2.13 (0.82)	1.66 (0.27)	0.004

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Appendix III: Table S1

TABLE S1 | Impact of age and gender on ultrasound-dermal thickness across skin sites.

Rodnan skin site	**Univariable linear regression Standardized β and p value**, N=140	**Multivariable linear regression Standardized β and p value,** N=140	**Univariable linear regression Standardized β and p value Males** (N=60) **vs Females** (N=80)
Face			
Age (years)	β = -0.322, p<0.0001	β = -0.368, p<0.0001	Males: β= -0.428, p=0.001 Females: β = -0.473, p<0.0001
Gender (male vs female)	β = -0.501, p<0.0001	β = -0.533, p<0.0001	-
Chest			
Age (years)	β = -0.014, p=0.874	Ns	Males: β = -0.137, p=0.3 Females: β = 0.057, p=0.613
Gender (male vs female)	β = 0.046, p=0.587	Ns	-
Abdomen			
Age (years)	β = -0.066, p=0.448	Ns	Males: β = -0.065, p=0.623 Females: β = -0.152, p=0.193
Gender (male vs female)	β = -0.334, p<0.0001	β = -0.347, p<0.0001	-
Upperarm			
Age (years)	β = -0.153, p=0.038	β = -0.193, p=0.01	Males: β = -0.200, p= 0.125 Females: β = -0.206, p=0.066
Gender (male vs female)	β = -0.513, p<0.0001	β = -0.486, p<0.0001	-
Forearm			
Age (years)	β = -0.223, p=0.009	β = -0.258, p=0.001	Males: β = -0.479, p<0.0001 Females: β = -0.079, p=0.489
Gender (male vs female)	β = -0.412, p<0.0001	β = -0.433, p<0.0001	-
Hand			
Age (years)	β = -0.217, p=0.01	β = -0.248, p=0.001	Males: β = -0.374, p=0.003 Females: β = -0.181, p=0.108
Gender (male vs female)	β = -0.43, p<0.0001	β = -0.448, p<0.0001	-
Finger			
Age (years)	β = -0.11, p=0.194	Ns	Males: β = -0.18, p=0.169 Females: β= -0.118, p=0.298
Gender (male vs female)	β = -0.393, p<0.0001	β = -0.403, p<0.0001	-

Rodnan skin site	**Univariable linear regression Standardized β and p value**, N=140	**Multivariable linear regression Standardized β and p value,** N=140	**Univariable linear regression Standardized β and p value Males** (N=60) **vs Females** (N=80)
Thigh			
Age (years)	β = 0.157, p=0.085	Ns	Males: β = -0.045, p=0.77 Females: β = 0.213, p=0.063
Gender (male vs female)	β = -0.381, p<0.0001	β = -0.365, p<0.0001	-
Leg			
Age (years)	β = -0.221, p<0.0001	β = -0.264, p=0.001	Males: β = -0.146, p=0.2777 Females: β = -0.45, p<0.0001
Gender (male vs female)	β = -0.393, p<0.0001	β= -0.420, p<0.0001	-
Foot			
Age (years)	β = -0.031, p=0.718	Ns	Males: β = -0.135, p=0.311 Females: β = -0.017, p=0.886
Gender (male vs female)	β = -0.431, p<0.0001	β = -0.438, p<0.0001	-

β values = standardized values