Influence of contextual factors and reliability of ultrasound skin measures in persons with systemic sclerosis and healthy controls

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Abstract

Objective
To examine the influence of contextual factors upon the evaluation of skin thickness and stiffness by ultrasound and to assess the reliability of these parameters.

Methods
Ultrasound dermal thickness (by B-mode, 18MHz) and skin stiffness (by shear-wave elastography, 9MHz) were assessed in persons with systemic sclerosis (SSc) and in healthy controls. The influence of contextual factors upon repeated measures was evaluated: (i) room temperature ($16\text{-}17^{\circ}C$ vs. $22\text{-}24^{\circ}C$); (ii) time of day (morning vs. afternoon), and (iii) menstrual cycle phase (menstrual vs. ovulatory). Differences were analysed using the related-samples Wilcoxon signed-rank test. Inter- and intra-rater reliability of ultrasound skin thickness and stiffness were evaluated in the 17 skin Rodnan sites of 20 persons with SSc and 20 healthy controls, under stable contextual conditions.

Results
A significant increase in ultrasound dermal thickness was observed at the leg in the afternoon vs morning, in both patients and controls. Similar observations were made for skin stiffness at the leg (in SSc) and at the foot (in SSc and controls) in the afternoon. No significant changes were observed in association with room temperature and menstrual cycle. Intra- and inter-rater-reliability was good to excellent for ultrasound dermal thickness and stiffness, both in SSc and healthy controls.

Conclusion
The timing of the ultrasound procedure within each day seems to influence the ultrasound measures at the legs and feet. Our study corroborates that ultrasound dermal thickness and skin stiffness are reliable domains to quantify skin involvement in SSc.

Key words
ultrasonography, elastography, systemic sclerosis, skin, dermis, thickness, stiffness

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Introduction

Skin involvement is a cardinal feature for the diagnosis and prognosis of systemic sclerosis (SSc) and is associated with worse functional ability and quality of life (1, 2). Measurement of skin involvement enables the prediction and monitoring of disease progression and treatment response (3, 4). However, the current gold standard for skin assessment, the modified Rodnan skin score (mRSS), used in clinical practice and clinical trials, presents several limitations (5).

Ultrasound is increasingly recognised as having advantages over mRSS, including its sensitivity to change, ability to identify early subclinical skin involvement and convergent validity against skin histologic findings (5-7). Gender and age have been found to influence ultrasound dermal thickness and skin stiffness (8-10), and preliminary normal reference percentile curves for these ultrasound measures have been proposed (11). The possibility that contextual factors, like room temperature, may influence these measurements is of crucial importance, but it has been very scarcely addressed (8-10, 12-15). Diurnal changes of dermal echogenicity (oedema) observed in previous studies (12-14), indicate that the hour of day may affect skin ultrasound parameters. One study reported that ultrasound skin thickness is influenced by sex hormone levels during the menstrual cycle (15). The possibility that room temperature may be relevant is suggested by common observation of tight skin upon cold exposure, by the importance of skin in body-temperature regulation and by its impact in vascular and cutaneous manifestations of SSc.

Prior studies evaluating the inter- and intra-reliability for ultrasound domains (5, 6), are limited by heterogeneity in their performance and report: different definitions used for skin thickness and skin sites assessed, different techniques for stiffness, and calculations based in mixed groups of patients and controls, among others.

These observations underline the need for evidence supporting current efforts to harmonise and consolidate the validity of skin US use in SSc, as highlighted in recent systematic literature reviews (5, 6) and recommendations (16).

This study aims at fulfilling some the identified knowledge gaps by: examining the influence of contextual factors upon skin ultrasound thickness and stiffness evaluation, and assessing the inter- and intra-rater reliability of these parameters, using currently recommended ultrasound methodology.

Materials and methods

Participants

Consecutive female persons with SSc (2013 ACR criteria) (17) attending the outpatient rheumatology and internal medicine clinic at Centro Hospitalar e Universitário de Coimbra (CHUC), Portugal, were recruited to four studies. Healthy female were recruited, among hospital staff, patients' family members and university students, respecting the following exclusion criteria: 1) diagnosis of any skin, connective tissue or rheumatic inflammatory disease, 2) history of insulin-treated diabetes, 3) history of anticancer chemo/radiotherapy treatments, 4) exposure to organic solvents, 5) past glucocorticoid treatment for more than 4 months, or 6) recent (<4 weeks) treatment with glucocorticoids, regardless of dose, clinical indication and timing.

Gender, age, medication, reproductive status (menopause, menstrual phase), disease duration, disease subset, mRSS and autoantibody profile, were collected from clinical files.

All participants provided signed inform consent prior to any study procedures. Ethical approval was obtained from the Ethics Committee of CHUC (CHUC-118-17).

Skin ultrasound measures

Skin assessment was performed using a Siemens ACUSON S2000 Ultrasound System HELX Evolution. Ultrasound dermal thickness and skin stiffness were evaluated in accordance with recent recommendations (16), as described in the Supplementary material. The present paper reports on four studies:

i) The influence of time of day

  Ultrasound evaluations were performed twice in the same day in each

Table I. Demographics and clinical characteristics of the participants included in the four studies: reliability, time of day, temperature, and menstrual phase variation.

			Female/male gender, n	Age*	Disease duration since first Raynaud*	mRSS**	Limited/ diffuse subset, n	Vasodilator treatment, Yes, n	Immunosup-pressive treat-ment, Yes, n, %
Reliability	Inter-	Patient	14/6	53.5 (21)	7.9 (9.0)	5.2 (0-14)	13/7	15 (75%)	10 (50%)
		Controls	16/4	64.0 (36)	-	-	-	0	0
	Intra-	Patient	18/2	59.0 (17)	9.5 (7.1)	5.1 (0-15)	5/15	10 (50%)	11 (55%)
		Controls	18/2	56.5 (14)	-	-	-	0	0
Contextual factors	Time of day	Patients	10/0	56.0 (16)	14.0 (13.0)	8 (0-22)	7/3	5 (50%)	4 (40%)
		Controls	10/0	39.5 (21)	-	-	-	0	0
	Room temperature	Patients	10/0	42.5 (18)	16.0 (13.2)	0 (0-18)	8/2	6(60%)	3(30%)
		Controls	10/0	64.0 (17)	-	-	-	0	0
	Menstrual phase	Patients	10/0	36.5 (5)	9.0 (5.3)	1.5 (0-13)	5/5	7(70%)	3(30%)
		Controls	10/0	31.0 (7)	-	-	-	0	0

*Median (IQR), years; **median (max-min).
All the participants were Caucasian, except for one patient that have participated in the inter-reliability study.

participant: between 08.30 and 10.30 a.m., and 8–9h later, 4.30 to 6:00 p.m. Room temperature was kept stable at 22–24ºC. Between the two evaluations, participants were free to perform their normal day routine.

ii) The influence of room temperature
Ultrasound evaluations were performed twice in the same patient. The first ultrasound evaluation was performed at warm room temperature (22–24ºC). The room temperature was brought down to the lower range (16–17ºC) with air conditioning and maintained stable. The patient was asked to rest for 15 minutes at this temperature and the second ultrasound evaluation was performed. Between the evaluations, the participant was kept in supine and relaxed position, with arms and legs uncovered. The acclimatisation time was interrupted if significant discomfort due to Raynaud/digital ulcers emerged.

iii) The influence of the menstrual phase
Ultrasound evaluations were performed on the same patients, on the menstrual (days 2–4) and on the ovulatory/early luteal (days 14–18) phase of the menstrual cycle. These days were chosen to account for the maximal difference in hormone levels. All these evaluations were performed in the morning period (8:30–12:00h) at 22–24ºC. All females included in this evaluation

had normal menstrual cycles (with or without contraceptive medication), as an inclusion criterion.

iv) Inter- and intra-rater-reliability of B-mode and shear-wave elastography
After four hours of training and protocol standardisation, two raters (rater 1, T.S., experienced in skin ultrasound for >5 years; and rater 2, M.S., >5 years of experience in musculoskeletal ultrasound, and with no previous experience in skin ultrasound) performed the ultrasound examinations at the 17 Rodnan skin sites, as described above. For inter-rater reliability, the ultrasound examination, was performed in the 17 mRSS sites, by the two raters, within an interval of ± 30 minutes. Intra-rater reliability was evaluated by rater 1 (T.S.), at two time-points, separated by 1 day to 4 weeks. Contextual factors were kept stable in all ultrasound evaluations (morning time, and room temperature 22–24ºC).

Studies i), ii) and iii) included 10 female SSc patients and 10 healthy controls. They were all performed by the same rater (T.S.), and comprised five skin sites: forearm, hand, finger, leg, and foot, at the dominant side, in the precise locations described by Moore *et al.* (18). This set of skin sites was selected because they are more often affected in early disease course (4). Study iv) included 20 SSc and 20 healthy controls.

Statistical analysis
For descriptive purposes, categorical variables are presented as absolute numbers with percentages, and continuous variables as medians and interquartile range (IQR). Comparison of paired data were performed with Wilcoxon signed-rank test. Calculations were performed using SPSS version 27.
Intra- and inter-rater reliability were calculated using the intra-class correlation coefficient (ICC) and the corresponding 95% confidence interval (95%CI). ICCs were calculated in absolute-agreement, two-way mixed models. ICC values <0.40 were taken as indicating poor, 0.40–0.59 fair, 0.60–0.74 good and values ≥0.75 excellent reliability (19).

Results
The demographic and clinical characteristics of the participants included in each of the four studies are given in Table I.

Time of day
A significant increase in dermal thickness values was observed between the morning and afternoon evaluations, at the leg 6.96% (IQR 3.39), $p=0.007$, and 7.39% (8.42), $p=0.018$, in SSc patients and in controls, respectively (Table II). A significant increase in skin stiffness was observed at the leg 18.21% (24.1), $p=0.047$, and foot 11.77% (12.8), $p=0.037$ in SSc patients, and at the foot

Table II. Skin ultrasound measures, dermal thickness, and skin stiffness, measured at the five Rodnan skin sites, in the morning (08.30-10.30am) and in the afternoon (4.30-6.00pm) periods of the same day.

	SSc patients (n=10)		Change from baseline, %	*p*-value	Controls (n=10)		Change from baseline, %	*p*-value
	Morning	**Afternoon**			**Morning**	**Afternoon**		
Dermal thickness (mm)								
Forearm	0.99 (0.20)	0.96 (0.19)	-1.64	0.767	0.87 (0.14)	0.87 (0.12)	2.40	0.878
Hand	0.84 (0.14)	0.87 (0.25)	0.35	0.953	0.74 (0.07)	0.74 (0.09)	0.99	0.778
Finger	0.87 (0.16)	0.88 (0.25)	-0.12	1.00	0.70 (0.07)	0.72 (0.06)	1.47	0.148
Leg	1.03 (0.18)	1.08 (0.20)	**6.96**	**0.007**	1.05 (0.17)	1.14 (0.17)	**7.39**	**0.018**
Foot	0.77 (0.09)	0.79 (0.10)	1.05	0.766	0.79 (0.71)	0.86 (0.08)	10.20	0.080
Skin stiffness (m/s)								
Forearm	1.78 (0.69)	1.87 (0.75)	2.24	0.477	1.36 (0.17)	1.39 (0.19)	0.78	0.838
Hand	2.02 (0.47)	2.17 (.78)	-0.25	0.475	1.73 (0.31)	1.76 (0.23)	1.03	0.635
Finger	2.94 (1.28)	2.91 (1.37)	-4.71	0.333	1.84 (0.27)	1.90 (0.23)	3.08	0.859
Leg	1.82 (0.59)	2.53 (0.90)	**18.21**	**0.047**	2.10 (0.47)	2.46 (0.79)	11.53	0.139
Foot	1.80 (0.59)	2.09 (0.39)	**11.77**	**0.037**	1.62 (0.32)	2.01 (0.29)	**14.42**	**0.021**

Skin ultrasound measures evaluated at the leg and foot are represented in Fig. S1 to S3 (available as supplementary online).

in controls 14.42% (15.98), *p*=0.021 (Supplementary Fig. S1-S3). No significant variations in the ultrasound parameters were observed in the other skin sites evaluated.

Room temperature
No significant changes in ultrasound measures (thickness or stiffness), were observed according to hot and cold room temperatures (Suppl. Table S1), in any of the five evaluated Rodnan skin sites.

Menstrual phase variation
There was no significant change in the skin ultrasound measures, between the menstrual (day 2–4) and the ovulatory/early luteal phase (day 14–18), in the five skin sites examined (Suppl. Table S2).

Inter- and intra-rater reliability of dermal thickness in controls and persons with SSc
The ICC for inter-rater reliability ranged, in the 17 different skin sites, from 0.85 (upper arm) to 0.96 (finger) in the SSc group, and from 0.82 (chest) to 0.96 (finger) in the control group. The ICC values for intra-rater reliability ranged from 0.84 (thigh) to 0.95 (abdomen) for SSc patients and from 0.83 (finger) to 0.94 (abdomen) in controls. Details of inter- and intra-rater reliability are presented in Supplementary Table S3.

Inter- and intra-rater reliability of skin stiffness in controls and persons with SSc
The ICC for inter-rater reliability ranged from 0.75 (chest) to 0.88 (thigh) in the SSc group and from 0.77 (forearm) to 0.98 (finger) in the control group. Intra-rater ICCs ranged, depending on skin site, from 0.78 (forearm) to 0.99 (finger) for persons with SSc and from 0.83 (face) to 0.98 (finger) in controls. Details of inter- and intra-rater reliability are described in Supplementary Table S4.

Discussion
The main findings of this report are that the time of day (morning *vs.* afternoon) may affect ultrasound measures, particularly in the leg and foot, whereas room temperature (within reasonable limits) and menstrual cycle phase have no significant impact. The influence of time of day is in line with previous studies (12, 20). Gniadecka *et al.* (12) demonstrated reduced echogenicity in the skin of the leg in older subjects, which was induced by water retention. Importantly, shear-wave velocity values seem to be a sensitive marker of skin oedema, as changes regarding time-of-day variation were more pronounced in stiffness than in thickness.
Conversely, variations in room temperature (hot *vs.* cold) as well as in the menstrual cycle phase, both putatively reasonable candidates to change skin

US measures, did not have a significant impact. Exposure to cold induces peripheral and cutaneous vasoconstriction and tightness, and we hypothesised that this might affect skin ultrasound parameters, at least in the acral body sites. However, this was not confirmed by our results. It may be argued that the time of acclimatation in cold temperature (15 minutes) was insufficient to have a significant impact. However, no changes were observed in the SSc group either, although four patients developed Raynaud or had increased pain due to digital ulcers in less than five minutes. The possibility that the menstrual cycle might influence skin ultrasound parameters is based on a previous ultrasound study (15). Isenbeiss *et al.* (15) reported that at the beginning of the menstrual cycle, when sex hormones are lowest, the skin is thinner compared to the middle of the cycle when oestradiol levels are highest, in women with a normal spontaneous menstrual cycle, *i.e.* without contraceptive medication. This was not confirmed in our study, in either patients or controls. It's noteworthy that about half of the participants were taking contraceptives which may have mitigated the variation in sex hormone levels, water retention and oedema (21). The small number of participants included in each category of this study hindered pairwise comparisons between the groups. Because there is a strong young female preponderance

reported in SSc, we believe that further studies considering this factor should be considered.

The need for a reliable tool to evaluate skin involvement is paramount in SSc. Our results are consistent and reinforce the report of in high reliability of ultrasound in the assessment dermal thickness, as described by a recent systematic review (6). Few studies have addressed the intra-and inter-rater reliability of stiffness evaluation using shear-wave elastography (5). Only one of these studies performed a full evaluation of the 17 Rodnan skin sites. The ICCs observed in our study corroborate these previous observations (5). Our ICCs for inter-rater reliability are better than those reported by Hou *et al.* (22) but, for the hand and finger, were slightly lower than those reported by Chen *et al.* using a 15MHz probe (5). We used a frequency probe of 9MHz with a lower image resolution, hampering the ability of accurately discern the epidermis-dermis interface. Although the recommendations advocate the use of a frequency probe ≥18mHz, these are not yet widely available for shear-wave elastography (5).

Strengths of our study include the adherence to a rigorous ultrasound evaluation, following recent recommendations on performance and report, with restricted experimental conditions to isolate the influence of the factors under study. Regarding limitations, this is a single centre study, with a homogeneous population (in terms of race/culture), limited to females and with a relatively small number of participants included in each study. These limitations reinforce that this is a preliminary study that needs to be further expanded to determine whether significant differences between groups occur in larger populations. Additionally, in this study we have only evaluated dermis, but it would be interesting if future work also evaluates the influence of contextual factors upon hypodermis. In a previous study (23), the use of a 70 MHz probe suggested that attention should be given to hypodermis, in particular in the very early phase of the disease.

This work addressed innovative and relevant research questions about the impact of contextual factors upon skin ultrasound measures in person with SSc and significantly contributes to the strength of existing data. It adds robust evidence on reliability, particularly regarding stiffness, with a full evaluation of all Rodnan sites, in both people with SSc and healthy controls. Our study showed that skin ultrasound is reliable even among sonographers with limited experience of skin ultrasound (rater 2), and that it can be learned with only minimal training, contributing to its feasibility. Further studies are warranted to investigate the impact of other potentially relevant factors on skin ultrasound measures such as, ultraviolet radiation, professional occupation, body mass index, peripheral oedema of cardiac/renal origin and medication (*e.g.* diuretics, glucocorticoids, contraceptives).

In summary, the time of day of ultrasound procedure seems to have influence on ultrasound measures, especially in the lower limbs, and this aspect should deserve attention when designing and reporting future trials. Good to excellent reliability was found for ultrasound dermal thickness and skin stiffness in all 17 Rodnan skin sites.

Take home messages
- Skin thickness and stiffness of the skin, as assessed by ultrasound tend to be higher in the afternoon *versus* morning at the legs and feet.
- Room temperature (16-17°C *vs.* 22–24°C) and the menstrual cycle do not influence these measures.
- High-frequency ultrasound and shear-wave elastography are reliable tools for skin assessment in systemic sclerosis

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