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EXTENDED REPORT

Longitudinal development of skin involvement and reliability of high frequency ultrasound in systemic sclerosis

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Background: Measurement of skin involvement is essential for diagnosis and prognosis in systemic sclerosis. Assessment by palpation skin score is the current method.

Objective: To explore high frequency ultrasound as an objective method to improve skin characterisation by combining measurements of skin thickness and echogenicity.

Methods: Skin thickness and echogenicity were measured at the proximal phalanx of the second digit, the back of the hand, the forearm, and the lower leg and, in order to separate limited (lSSc) from diffuse SSc (dSSc), at the chest in 16 patients with early disease and in 16 controls. Measurements were repeated in each patient three or four times with an interval of 1–2 years. Ultrasound measurements of skin thickness were compared with the palpation skin score.

Results: Compared with controls, the skin was thickened on the phalanx, hand, forearm, and chest of patients with dSSc, but not patients with lSSc, at the 1 year examination. In dSSc the degree of thickening tended to diminish with time, and at 4 years, thickness was significantly decreased on the forearm and chest compared with the 1 year measurements. Low reflectancy was most pronounced at the 1 year evaluation on the forearm, hand, and phalanx in dSSc, and on the phalanx in lSSc.

Conclusion: Separate measurements of skin thickness and echogenicity by high frequency ultrasound add a new dimension to the assessment of skin involvement in SSc, and this seems to be an objective non-invasive tool for use in the study of disease development and in clinical trials.

Skin thickening and tightness are characteristic manifestations of systemic sclerosis (SSc, scleroderma) and the only major diagnostic criterion.¹ The pathophysiology consists of vascular damage, inflammation, and excessive deposition of extracellular matrix by fibroblasts. The extent of skin involvement in early disease permits the diagnostic distinction between limited (lSSc) and diffuse SSc (dSSc).² Three phases of skin involvement can be identified: an early oedematous phase associated with increased amounts of interstitial fluid, an indurative phase during which newly synthesised collagen is deposited in the skin, and an atrophic phase in which thinning of the abnormal skin may occur.³

In 1979 Rodnan introduced the semiquantitative scoring technique of skin palpation to characterise and quantify skin involvement in SSc.⁴ It is today in general use with minor modifications,^{5–8} despite the shortcomings of observer variation^{7–8} and inability to distinguish between the features skin thickness and tightness. In 1979 Alexander and Miller introduced a new non-invasive method for measurement of skin thickness by 15 MHz ultrasound.⁹ The determination of skin thickness with 10–15 MHz is a reproducible method,^{10–11} but requires easily identifiable interfaces. High frequency (20 MHz) ultrasound offers considerably better resolution, which makes it possible to distinguish dermis from subcutaneous fat, and to measure skin thickness regardless of the underlying tissue. The development of very high frequency, high resolution equipment has made cross sectional (two dimensional) images possible,¹² which not only allow the determination of skin thickness but also a qualitative assessment of the skin composition. Dermal echoes are in most body regions many and variable. They originate from the well organised fibre network, which is also responsible for the tensile properties of the skin. Conditions, which compromise this network, cause low echogenicity and reflect, for example, subepidermal increase of interstitial fluid in oedema.¹³ High frequency ultrasound has been used in

several skin diseases¹⁴ but only in limited amounts in localised¹⁵ and systemic scleroderma.^{16–19} This report examines the performance of a standardised high frequency ultrasound protocol in longitudinal observations of patients with early phases of both types of SSc. The results were compared with the Rodnan score and with ultrasound measurements in healthy controls.

PATIENTS AND METHODS

Patients

Sixteen patients, who all fulfilled the American College of Rheumatology criteria for the classification of scleroderma,¹ and 16 controls were selected for the study. The controls comprised sex matched healthy subjects randomly selected from the Swedish population register. Eight patients had skin thickening restricted to the extremities and face (lSSc) and eight also had skin sclerosis on the trunk (dSSc).² Disease duration, defined from the beginning of skin involvement, was 1 year or less in 13 patients or 2 years or less in three patients. Table 1 shows the demographic features of patients and controls.

Ultrasound

Skin thickness and echogenicity were measured with a high frequency ultrasound scanner (Dermascan, Cortex Technology, Denmark), in which a 20 MHz transducer was mounted in a water chamber. The chamber window was covered with a disposable plastic membrane. A conductive gel was used to ensure satisfactory contact between the crystal face and the skin. The transducer was placed perpendicular to the skin. Two scans were obtained of the tissue: a one dimensional A mode image with different echoes defining the interfaces between epidermis, dermis, and subcutis, and a two dimensional B mode image with different colours reflecting the differing echogenicities of the skin (fig 1). The quality criterion adopted for acceptance of an ultrasound

Table 1 Demographic features of patients with SSs and of controls

	Systemic sclerosis		Controls
	Diffuse	Limited	
Number (female)	8 (7)	8 (7)	16 (8)
Age (years)	45 (26–67)*	52 (33–68)*	44 (27–70)*
Duration ≤ 1 year	8	5	
Duration ≤ 2 years		3	
TSS (points)	27 (12–32)	7 (4–19)	
Anti-topoisomerase-1	4	1	
ACA	1	4	

*Median (range).

TSS, total skin score; ACA, anticentromere antibody.

image was the reproduction of echoes, which defined the interfaces between the epidermis, dermis, and subcutis by the A mode, and a correlated B mode image, which showed the echogenicity of the epidermis, dermis, and subcutis. The measurement was made at a site in the B mode image where the demarcation lines between the epidermis, dermis, and subcutis were parallel, and the echoes of the corresponding A

mode image distinct. The echogenicity in the dermal region was represented on an arbitrary scale (0–255 pixels), and by outlining a block of skin the mean echogenicity was estimated for a selected region^{12–16}. The measurements of skin thickness and echogenicity which are given in the results stand for the epidermis plus dermis.

All measurements were made before noon. The measuring points were selected in order to distinguish skin involvement in lSSc and dSSc, and to monitor disease development over time. Measurements were made at each of five skin sites—over the dorsal aspect of the interarticular portion of the proximal phalanx of the right second finger (*phalanx*), over the area (valley) between the metacarpophalangeal joints II and III of the right hand (*hand*), over the dorsal aspect of the right forearm 3 cm proximal of the wrist (*forearm*), over the lateral aspect of the leg 12 cm proximal of the ankle joint (*leg*), and over the sternum 2 cm distal from the upper part of the manubrium (*chest*)—and repeated in each patient three or four times with an interval of 1–2 years.

Skin score

Skin involvement was determined by palpation of 17 anatomical sites (face, fingers, hands, forearms, upper arms, chest, abdomen, thighs, legs, and feet) and scoring on a 0–3

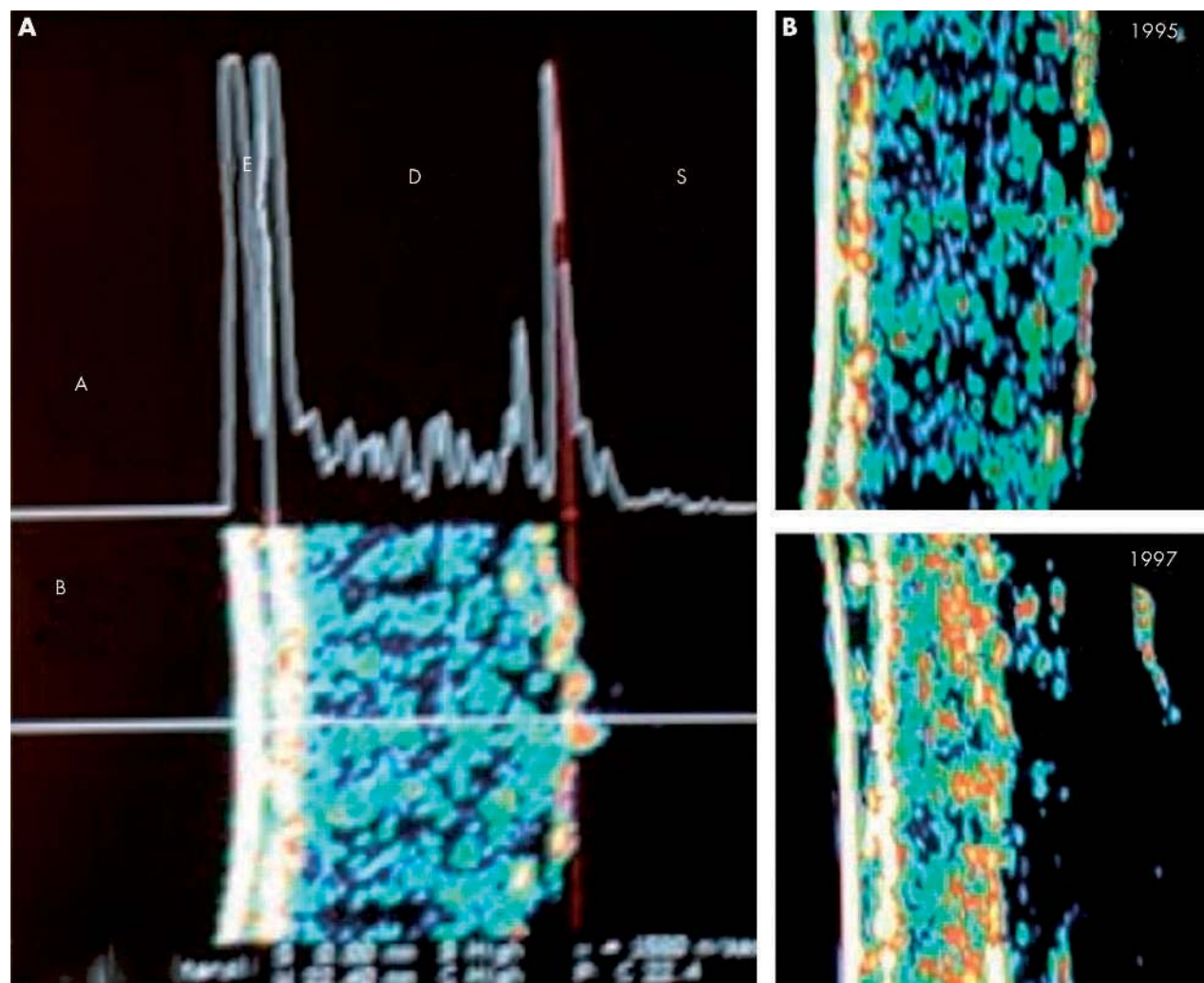


Figure 1 (A) Skin thickness and echogenicity by high frequency ultrasound. A, A mode image of the forearm skin. Echoes defining the epidermis (E), dermis (D), and subcutis (S) are marked. B, B mode image of the forearm skin with the epidermis, dermis, and subcutis. (B) Skin thickness and echogenicity in a patient with dSSc. At 1 year's disease duration skin thickness was 2.5 mm and skin echogenicity 16 pixels. Two years later skin thickness had decreased to 1.3 mm, while skin echogenicity had increased to 36 pixels.

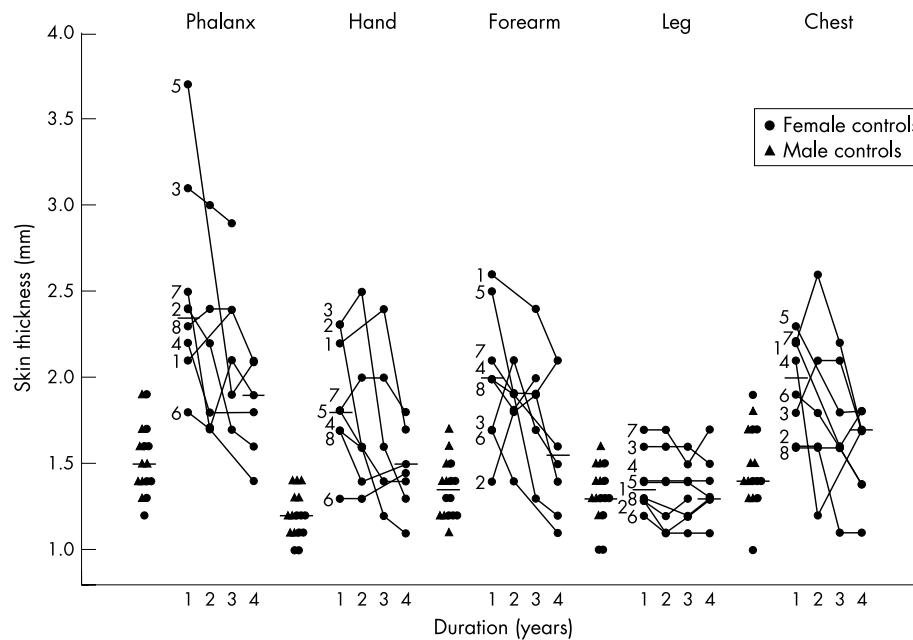


Figure 2 Skin thickness measured by high frequency ultrasound over the phalanx, hand, forearm, leg, and chest at 1–4 years' disease duration in patients with dSSc.

scale, where 0 = normal skin, 1 = slight thickening, 2 = moderate thickening, and 3 = hidebound skin sclerosis. The scores for all sites were summed to give a total score, with a maximum possible score of 51. The palpation was performed by one of two assessors (AS/ÅÅ), both well trained in skin palpation and unaware of the result of the ultrasound assessment of the patient.

Immunological analyses

Anti-topoisomerase I antibody positivity (scleroderma-70 antibody) was detected by immunodiffusion, and anti-centromere antibody positivity by immunofluorescence using an HEp-2 substrate (performed by the Department of Clinical Immunology, University Hospital, Lund, Sweden).

Statistics

The significance of differences between patients and controls was calculated with one way analysis of variance, followed by

multiple comparisons using Tukey's method. Associations between ultrasound measurements and skin scores were calculated with the Pearson correlation test.

Reproducibility was measured by the mean of the absolute differences between the observers and intraclass correlations (ICC). The measurements were derived from the first visit of the patients and from all controls. All measurements were performed before noon by two trained observers (MW and ÅÅ), who each obtained two ultrasound images of all investigated areas. Both observers measured thickness and echogenicity of all four images (table 2). Interobserver and intraobserver variability were calculated for both MW and ÅÅ with the same results. Intraobserver variability of ultrasound measurements in table 3 are calculated from measurements by MW, who measured thickness and echogenicity of all pictures at two different occasions, and interobserver variability by MW and ÅÅ, who independently measured the thickness and echogenicity of every image.

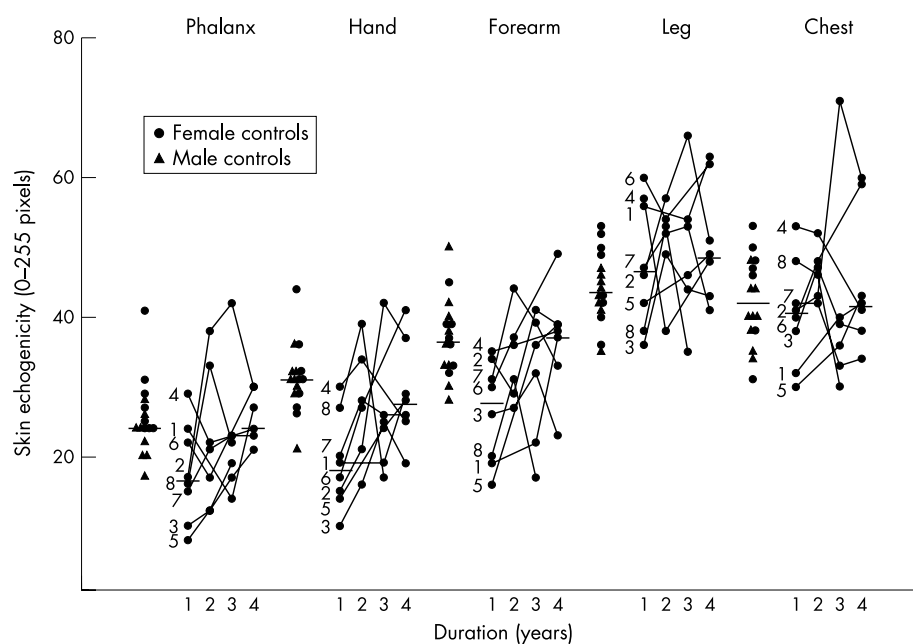


Figure 3 Skin echogenicity measured by high frequency ultrasound on the phalanx, hand, forearm, leg, and chest at 1–4 years' disease duration in patients with dSSc.

Table 2 Skin thickness and echogenicity in patients with SSc and controls at the 1 year measurements

	Systemic sclerosis		Controls	Differences between dSSc and lSSc (p value)
	Diffuse	Limited		
Thickness				
Phalanx	2.5 (0.6)***	1.9 (0.3)	1.5 (0.2)	0.02
Hand	1.9 (0.4)***	1.4 (0.4)	1.2 (0.1)	0.02
Forearm	2.0 (0.4)***	1.5 (0.2)	1.3 (0.2)	<0.01
Leg	1.4 (0.2)	1.3 (0.2)	1.3 (0.2)	NS
Chest	2.0 (0.3)***	1.5 (0.2)	1.5 (0.2)	<0.01
Echogenicity				
Phalanx	17.5 (7.1)**	19.3 (6.0)*	27.3 (6.0)	NS
Hand	18.9 (6.7)**	28.0 (13.6)	31.8 (5.3)	NS
Forearm	26.4 (7.2)**	39.8 (9.8)	37.6 (5.6)	<0.01
Leg	47.8 (9.0)	50.2 (6.4)	44.0 (5.5)	NS
Chest	40.5 (7.6)	47.6 (7.0)	42.5 (6)	NS

*Significantly different from controls $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
Results are shown as mean (SD).

RESULTS

At the first evaluation the skin was thicker than that of the controls on the phalanx, hand, forearm, and chest in patients with dSSc, while synchronous measurements showed decreased echogenicity of the phalanx, hand, and forearm skin (table 2). Compared with the first evaluation, repeated measurements at 2–4 years showed diminished skin thickness in most patients, and after 4 years the skin on the forearm and chest was significantly decreased ($p < 0.05$, fig 2). Skin echogenicity also changed, but was significantly increased after 4 years only on the skin of the hand ($p < 0.05$, fig 3).

Figure 4 shows serial measurements of skin thickness and skin echogenicity in a patient with dSSc. At 1 year skin thickness on the phalanx and hand was above the normal range, concurrent with a low skin echogenicity. Compared with the 1 year evaluation measurements, increasing echogenicity was seen at 2 and 3 years in all areas except the forearm. At the 4 year evaluation skin thickness was normal or decreased in all areas, concurrent with a decrease in skin echogenicity.

Most patients with lSSc had thickened skin on the phalanx and hand throughout the study (table 2, fig 5), while skin echogenicity on the phalanx was decreased compared with controls at the first evaluation (table 2, fig 6).

Table 3 outlines reliability data of the ultrasound measurements. Although both the interobserver and intraobserver reliability varied depending on the anatomical site, the results indicate that the precision of measurements of skin thickness and echogenicity is reproducible between technicians (MW/ÅÅ) and within technicians (MW). Measurements of skin thickness and echogenicity were not related to regional skin scores in patients with dSSc, except for skin echogenicity of the hand ($p < 0.01$) at the 3 year evaluation. In patients with lSSc skin thickness was related to the regional score of the hand ($p < 0.05$) at the 2 year measurement, to regional scores of the phalanx and hand ($p < 0.01$) at 3 years, and to scores of the forearm ($p = 0.02$) and chest ($p < 0.001$) at 4 years.

The controls were not followed up. It has been presumed that a number of variables—namely, race, age, sex, anatomical site, and time of the day may be relevant for the outcome of measurements of skin thickness. Although the number of patients was small, no differences of age were found for either thickness or echogenicity among the controls. This is accordance with results by de Rigal and coworkers,²⁰ who by ultrasound imaging observed a phase of maturation up to 15 years of age, after which skin thickness

did not vary significantly until after the seventh decade. Nor did the present study show differences in thickness and echogenicity between male and female controls (figs 2–5). On the contrary, table 2 shows differences due to anatomical site both with respect to thickness and echogenicity.

DISCUSSION

Skin involvement in scleroderma reflects internal organ pathology. The extent of early skin involvement distinguishes the two major forms of SSc and correlates with prognosis.²¹ Therefore there is a need for a reliable technique not only to assess the extent and degree of skin thickening but also to recognise different stages of the disease.

The Rodnan skin score⁴ and its modifications^{5–8} employs a qualitative rating scale of skin changes on palpation of multiple body areas, and is a semiquantitative tool validated for clinical assessment and research. A relationship exists between the weight of a forearm skin biopsy and both regional³ and total thickness score,²² but interestingly not with a skin oedema score.²² This illustrates the limitations of clinical palpation to distinguish different stages of the disease.

The emergence of high frequency ultrasound technology has made cross sectional images possible, which allow not only precise measurement of skin thickness but also qualitative assessment of the skin in vivo. The temporal development of truncl involvement has not been investigated in detail, despite its importance for distinction between the subsets. Skin, which appears to be unaffected among patients with scleroderma, might nevertheless be abnormal. Despite absence of skin changes by palpation and histological examination Ihn *et al* reported significantly increased thickness by ultrasound of both clinically affected and unaffected skin in patients with SSc compared with controls.¹⁷ This discrepancy might be due to the inability to recognise oedema by palpation, and to loss in skin thickness at biopsy.

By high frequency ultrasound it seems possible to detect different stages of the disease in different investigated areas. This is exemplified in fig 4, which shows serial measurements of skin thickness and echogenicity in a patient with dSSc. At the first evaluation skin thickness on the phalanx and hand is higher than normal, while skin echogenicity is lower than normal. Because an increase in interstitial fluid is a known cause of low reflectancy,¹³ the thickening might be due to oedema in these areas. Compared with the 1 year evaluation, measurements at 2 and 3 years show increased echogenicity in all areas except the forearm. This may be due to

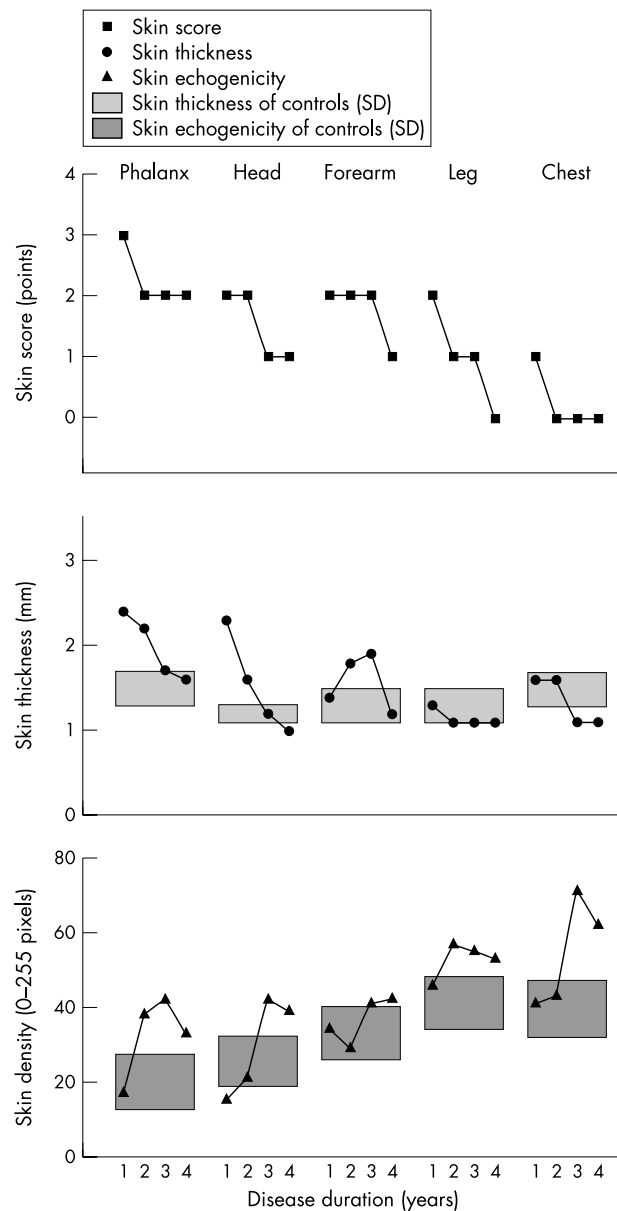


Figure 4 Skin thickness assessed by clinical palpation with rating on a 0-3 scale (where 0=normal skin, 1=slight thickening, 2=moderate thickening, 3=thickened immobile skin) and measurements of skin thickness and echogenicity by high frequency ultrasound at 1-4 years disease duration in a patient with dSSc.

replacement of interstitial fluid by newly synthesised collagen in these locations,²³ and a change of skin involvement from the oedematous to the indurative stage of the disease. At the 4 year evaluation, skin thickness is normalised or decreased in all areas, concurrent with a decrease in skin echogenicity, which might indicate that the overproduction of matrix components has regressed.

In the present study this is further illustrated by repeated measurements of skin thickness and echogenicity in patients with dSSc, who at the first evaluation had skin echogenicity on the chest which was not different from controls, while skin thickness on the same area was increased (table 2). This might indicate that the chest skin has already reached the indurative stage, while decreased echogenicity and increased thickness of other investigated areas points to a continuous oedematous phase of the disease.

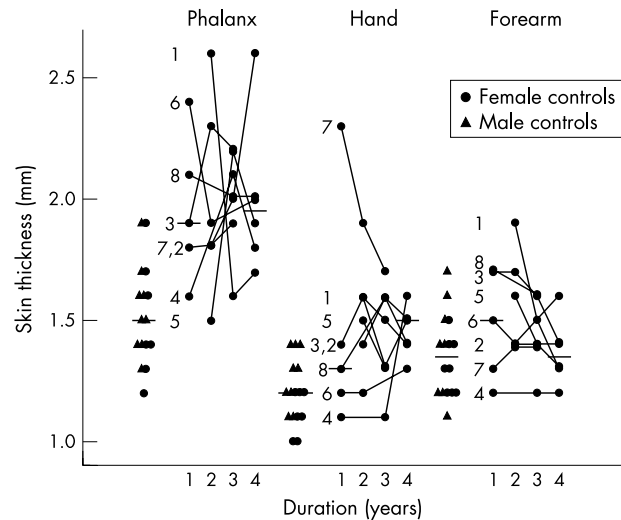


Figure 5 Skin thickness measured by high frequency ultrasound on the phalanx, hand, and forearm at 1-4 years' disease duration in patients with SSs.

Clinical palpation may not detect skin thickness but rather changes of skin texture, which might be due to either increased amounts of interstitial water or matrix components. This may explain the absence of association between the skin score and ultrasound measurement in most locations investigated, and is concordant with comparisons between skin scoring and durometer readings.²⁴

The reliability of the ultrasound technique allows recognition of small and serial changes in the extent and nature of skin involvement. Although the number of patients is small it seems possible to visualise the transition from an oedematous to an indurative phase by combining measurements of skin thickness and skin echogenicity and thus facilitate estimation of the disease stage. This may be important in characterisation of the natural course of the disease and in clinical studies, which has been shown in morphoea,²⁵ but not yet in SSs.

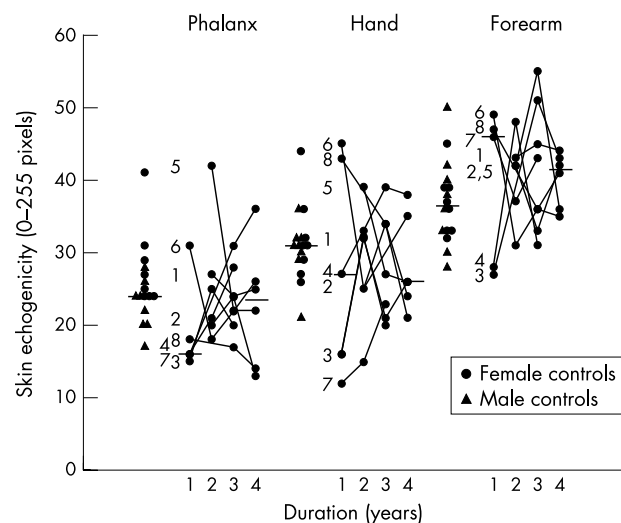


Figure 6 Skin echogenicity measured by high frequency ultrasound on the phalanx, hand, and forearm at 1-4 years' disease duration in patients with SSs.

Table 3 Reliability data of ultrasound measurements

	Interobserver variation				Intraobserver variation			
	Thickness		Echogenicity		Thickness		Echogenicity	
	Mean (mm)	ICC	Mean (pixel)	ICC	Mean (mm)	ICC	Mean (pixel)	ICC
Phalanx	0.15	0.66	1.3	0.98	0.05	0.95	2.6	0.83
Hand	0.06	0.83	1.1	0.97	0.04	0.92	2.7	0.81
Forearm	0.08	0.83	0.6	0.99	0.05	0.96	2.4	0.88
Leg	0.07	0.88	1.4	0.92	0.03	0.97	2.7	0.82
Chest	0.10	0.84	0.8	0.99	0.04	0.98	2.7	0.87

ICC, Intraclass correlation.

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