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Objective quantification of burn scar stiffness using shear-wave elastography: Initial evidence of validity

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ABSTRACT

Shear-wave elastography (SWE) is an ultrasound based technology that can provide reliable measurements (velocity) of scar stiffness. The aim of this research was to evaluate the concurrent validity of using both the measured velocity and the calculated difference in velocity between scars and matched controls, in addition to evaluating potential patient factors that may influence the interpretation of the measurements.

Methods: A cross-sectional study of 32 participants, with 48 burn scars and 48 matched contralateral control sites were evaluated with SWE, the Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS) tactile sub-scores.

Results: Spearman's rho demonstrated high correlations ($r > 0.7$) between the measured scar velocity and both the POSAS and VSS pliability sub-scores, whereas moderate correlations ($r > 0.6$) were found with the calculated difference in velocity. Regression analysis indicated that the association of increased velocity in scars, varied by length of time after burn injury

Abbreviations: SWE, shear-wave elastography; VSS, Vancouver Scar Scale; POSAS, Patient and Observer Scar Assessment Scale; ROI, region of interest; HTS, hypertrophic scar.

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and gender. Body location and Fitzpatrick skin type also demonstrated significant associations with velocity, whereas age did not.

Conclusion: SWE shows potential as a novel tool to quantify burn scar stiffness, however patient factors need to be considered when interpreting results. Further research is recommended on a larger variety of scars to support the findings.

1. Introduction

Increased tissue stiffness is associated with diseased and dysfunctional tissue, including stiff, thick pathological scars. Tissue stiffness is not a static tissue state, it is continuously regulated and actively modified by mechanosensitive cells including fibroblasts and stem cells. These cells sense various mechanical stimuli such as strain, compression, shear and osmotic forces [1], and respond by altering cell behaviors and extracellular matrix remodeling, which then alters tissue stiffness [2]. Intra and extracellular mechanical stimuli affect all phases of wound healing, influencing the quality of the resultant scar [3]. Furthermore, mechanical forces are used frequently in therapies to minimize scar formation, including compression, massage, exercise, vacuum assisted devices and stretch. However, the mechanisms underlying these therapies are not well understood. Understanding the role that mechanical forces play in wound healing, scar formation, and scar minimizing therapies in humans has been limited due to the lack of an objective, non-invasive assessment tool to quantify skin stiffness.

The evaluation of scar stiffness is incorporated into subjective scales, such as the Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS) under the term 'pliability'. Subjective evaluation is completed by manually manipulating the scar tissue with fingers to provide an overall score of the palpable stiffness and extensibility of scars [4–8]. Pliability, however is a collective term which encompasses a few fundamental tissue properties including elasticity, stiffness and viscoelasticity. Objective measurement tools can evaluate each of the tissue properties independently providing more accurate measurements that may relate directly to tissue physiology [9–11]. Objective tools used to evaluate scar mechanical properties were extensively reviewed recently by Lee (2016) [12]. All these assessment tools apply various macro-level forces, creating large tissue displacements which provide a measurement dependent on the temporal and spatial qualities of the force, and the area of tissue evaluated [13]. These tools typically don't provide a quantitative measurement of the tissue property, such as a Young's or shear modulus, and are therefore only able to provide within person comparisons and not between person comparisons. Furthermore, functional limitations of these tools include low levels of repeatability [14,15], low to moderate correlation with subjective clinical assessments [10,11] and a reduced capacity to evaluate firmer scars due to the degree of force required to deform the tissue [15,16].

Shear-wave elastography (SWE) can provide objective quantification of tissue stiffness using acoustic radiation forces (ARF) (high intensity sound waves) to generate focused microscale displacements of tissue. Based on an ultrasound platform, SWE uses multiple, rapid ultrasound images to capture shear-wave propagation away from the region of force excitation. Shear-wave velocity directly correlates to tissue stiffness and from this, an estimate of the shear modulus can be calculated [17,18]. SWE is widely used to quantify tissue stiffness in the liver [19], breast [20], and musculoskeletal disorders [21], however it is relatively new to dermatology. Our previous research demonstrated excellent intra-rater, inter-rater and test-retest reliability when the methods detailed in this study were used to evaluate both skin and scars. In addition, we were able to discriminate between skin, non-pathological and pathological scars as defined by VSS height and pliability scores, and we were able to evaluate burn scars ranging in stiffness from VSS pliability scores zero to five [22].

Furthermore, our previous study developed preliminary shear-wave velocity cut-off values to classify pathological scars from non-pathological scars i.e. scars that have returned to 'normal' height (VSS height = 0) and 'normal' pliability (VSS pliability = 0), and from skin [22]. However, a large range in the velocities of normal skin (range = 1.06 m/s–4.94 m/s) contributed to a lower than desired sensitivity and specificity between skin and non-pathological scars (2.09 m/s, 70% sensitivity 76% specificity) and between non-pathological scars and pathological scars (2.22 m/s 70% sensitivity and 88% specificity). Intrinsic person factors known to influence skin stiffness include body location [23,24], age [25,26], gender [27] and Fitzpatrick Type [28]. It was hypothesized that these patient factors were influencing velocity, leading to the misclassification of skin and scars. Therefore, the aims of this study were to firstly calculate the difference in velocity between scars and matched controls (diff-velocity), secondly to evaluate the concurrent validity between the measured shear-wave velocity, the diff-velocity, and both the POSAS and VSS pliability sub-scores, and thirdly to explore if the above patient factors were associated with shear-wave velocity.

2. Material and methods

2.1. Study design

This was a cross-sectional, exploratory study approved by the South Metropolitan Health Services Human Research Ethics Committee (EC00265) in Western Australia.

2.2. Participants

Participants were recruited between May and December 2018 from the Burns Outpatient Clinic, at Fiona

StanleyHospital, Perth Western Australia. Participants 18 years or over, with at least one burn scar undergoing active treatment and a matched non-injured contralateral skin site, were invited to participate. To maximize the recruitment of participants with palpable levels of pathological scarring, participants were excluded if their most severe scar scored zero on both the pliability and height constructs of the Vancouver Scar Scale (VSS). In addition, participants were excluded if they were being treated for open wounds, were not able to provide written consent due to psychological, cognitive or language impairments or if they had a pre-existing dermatological condition e.g. dermatitis, eczema, or psoriasis.

2.3. *Shear-wave elastography (SWE)*

An ACUSON S3000 (Siemens Heathineers Pty Ltd., Australia and New Zealand) ultrasound system in the Virtual Touch IQ (VTIQ) elastography mode was used in this study. A 9L4 linear transducer is used to induce micrometer-scale displacements of tissue within a targeted area using multiple ARF push pulses [29]. The tissue displacement generates shear-waves that propagate through the surrounding tissues in an orthogonal direction to the push pulse. Multiple detector beams track the shear-wave propagation, providing an estimate of the velocity, with higher velocities indicating stiffer tissue [17]. The maximal shear-wave velocity detectable is ten meters per second (m/s). Built-in software then calculates the shear modulus from the measured shear-wave velocity, reported in Kilopascal (KPa). However, the software used on the S3000 to estimate the shear modulus has been optimized for deep tissue imaging, rather than for skin imaging, so as per current recommendations, only velocity measurements were used for statistical analysis in this study [17,30]. The shear modulus will still be presented in this paper for comparison purposes because some studies only report the shear modulus.

A two-dimensional, SWE color image, called an elastogram (Fig. 1) is overlaid on the B-mode ultrasound image to visualize tissue stiffness. The color pixels are coded to represent the different levels of stiffness, with red pixels (higher velocity) representing stiffer regions and blue pixels (lower velocities) representing softer regions. Both the shear-wave velocity and shear modulus measurements are obtained by placing

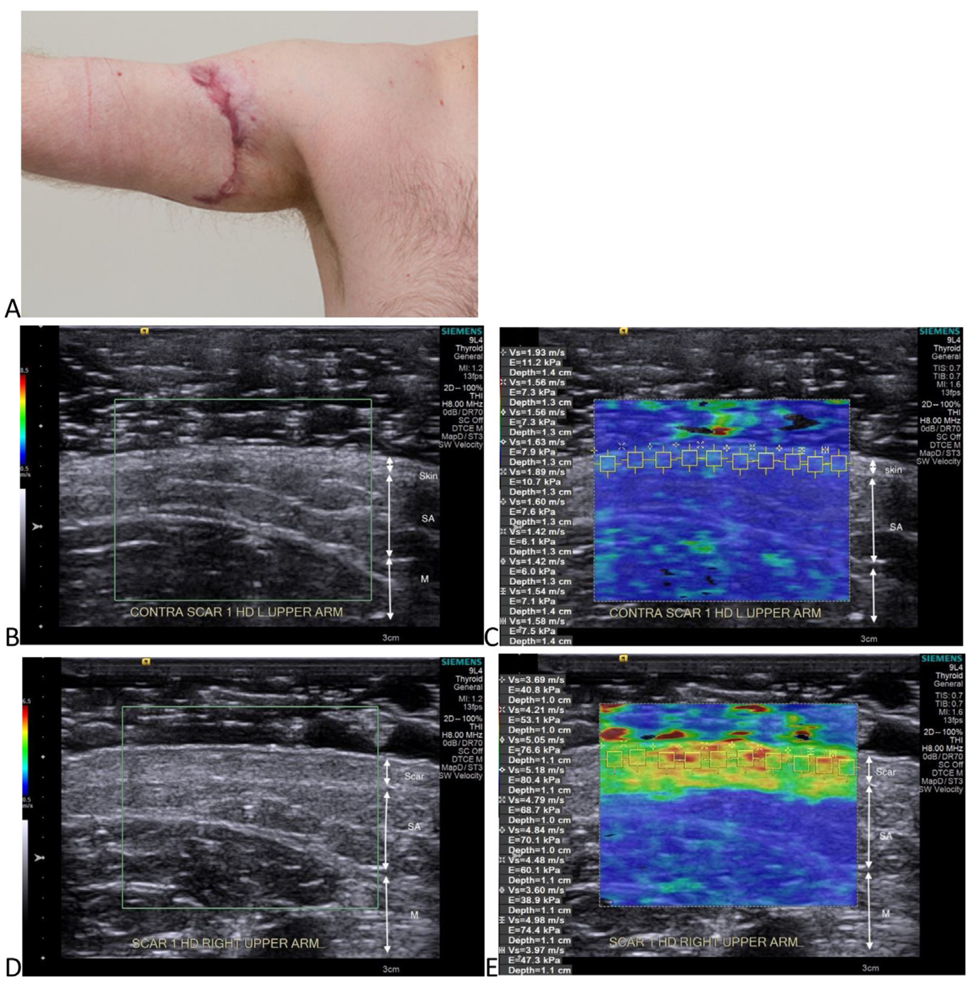

Fig. 1 – A) Photo of a 14 month old burn scar of on the upper arm of a 23 yo male. B) Ultrasound of the contralateral healthy skin. C) the corresponding elastogram with an average velocity of 1.61 m/s (7.87 kPa). D) The ultrasound of the burn scar. E) The corresponding elastogram of the burn scar with an average velocity of 4.48 m/s (61.04 kPa). [SA=subcutaneous adipose, M=muscle].

sampling boxes (1.5 mm^2) onto the image. The quality of the elastograms can be assessed using built-in software which provides a separate image indicating adequate propagation of the shear waves.

2.4. *Subjective scar assessments*

2.4.1. *The Vancouver Scar Scale (VSS)*

The VSS is a validated scar assessment tool [31–33], the Baryza and Baryza version [33] was used in this study (Table 1). The VSS evaluates four clinical characteristics of scarring including vascularity, pigmentation, pliability and height [34]. The VSS has been used numerous times to evaluate the validity of other scar assessment tools [10,35–40]. However, the VSS scoring scale has been criticized for being insensitive to change and having inconsistent ranges (height 0–4, pliability 0–5, vascularity 0–3, and pigmentation 0–3) limiting the ability to provide a summated total score [9,41,42]. Therefore the scores of each characteristic are recommended to be used independently [31]. The height and pliability sub-scores have demonstrated independent construct validity against objective assessment tools [43] and were used in this study. A score >0 in the height characteristic can be used to classify a scar as hypertrophic (HTS) [31].

2.4.2. *The Patient and Observer Scar Assessment Scale (POSAS)*

The POSAS is an alternative valid and reliable scar scale [36,44]. The Observer POSAS evaluates six scar characteristics including pliability, thickness, relief, pigmentation, surface area and vascularity (https://www.posas.org/the-posas/the-scale/). Each characteristic is scored on a ten-point numerical rating scale, which makes the POSAS more sensitive to change compared to the VSS. The scores of the POSAS can be summated for use as a total score [36] or can be used individually [11,44,45]. Concurrent validity was moderate ($r < -0.53$) between the POSAS pliability sub-scale and objective measurements of skin extension, pliability and elasticity using the Cutometer® in burns patients [46]. In addition, the POSAS pliability sub-score taken at three months after burn demonstrated predictive validity of scar quality at 18 months after burn [11]. The three tactile characteristics: pliability, thickness and relief were evaluated in this study.

Table 1 – Vancouver Scar Scale.

Skin characteristics	Parameters
Pliability	
0	Normal
1	Supple
2	Yielding
3	Firm
4	Banding
5	Contracture
Height	
0	Normal-flat
1	>0 to 1 mm
2	>1 to 2 mm
3	>2 to 4 mm
4	>4 mm
Vascularity	
0	Normal
1	Pink
2	Red
3	Purple
Pigmentation	
0	Normal
1	Hypopigmentation
2	Mixed pigmentation
3	Hyperpigmentation

2.5. *Personnel*

Two senior sonographers participated in and supervised this study. MaZ had 31 years of sonography and 11 years of elastography experience, and SA had 10 years of sonography and 5 years of elastography experience. An Occupational Therapist (HD) with over 20 years of experience in scar assessment and treatment completed the clinical scar assessments prior to SWE. Ten hours of SWE training was completed by the therapist and the competency of image acquisition was evaluated with excellent intra-rater (ICC = 0.98) and inter-rater (ICC = 0.96) reliability [22]. MaZ independently reviewed the elastograms for presence of artifact.

2.6. *Procedure*

Recruitment was commenced at six weeks after injury or surgical procedure to coincide with the routine follow-up appointments. Informed, written consent was obtained from invited participants meeting the inclusion criteria. The participant was asked to remove any pressure garments, and was positioned on a hospital bed for at least five minutes in a temperature-controlled (22 ± 1 °C) room. Participants had up to three scar sites with matched contralateral non-injured skin sites identified to act as within person controls. Minimal differences in skin stiffness has been demonstrated between the two sides of the body on healthy participants using SWE, thereby indicating matched contralateral sites can act as a control for skin conditions [24]. The size of each site evaluated was 30 mm × 10 mm, the same size as the contact surface of the ultrasound transducer. The participant was lying on the bed in a comfortable position so that the scar was positioned horizontally, and the surrounding joints positioned in slight flexion to minimize tension/stretch on the scar. This position was mirrored for the contralateral skin site.

The therapist completed the subjective assessments for each scar site, then used a transparent film to record a tracing of the scar, noting significant landmarks which were used to locate the matched control site. The imaging protocol was developed by our group to optimize SWE of burn scars [22]. Briefly, a custom-made silicone gel-well is placed onto the scar (or skin) and filled with transmission gel (ecoultragel, Pirrone srl) to a minimum depth of 10 mm. The ACUSON S3000 was switched to VTIQ elastography mode, and the transducer was placed onto the gel, horizontal to the skin surface with minimal pressure to avoid compression artefact.

The maximum focus depth was set to 30 mm, and the elastography region-of-interest (ROI) was pre-set at 20 mm × 25 mm. The ROI was placed to ensure the gel, dermis and subcutaneous adipose were clearly within the ROI. The visual

display was set to 0.5–6.5 m/s, however, for tissues that were extremely stiff the scale was adjusted to 10 m/s. This does not alter the measured velocity, only the visual display. A B-mode ultrasound was taken, ensuring a clear entry echo, then the elastogram was acquired immediately thereafter. Ten sampling boxes were placed onto the elastogram, just below the entry echo to obtain the quantitative measurements of stiffness. Elastograms for each site were repeated up to five times to ensure three elastograms with minimal artefact were acquired. Velocity measurements from the first elastogram were used in the analysis.

2.7. *Statistical analysis*

Data analysis was conducted using Stata version 14.0 (StataCorp LP, 2015). The raw velocity (ten measurements per elastogram), mean measured velocity and the mean diff-velocity (calculated mean difference in velocity between scars and matched controls) were used in the analyses.

The mean, standard deviation (SD) and the range of measured velocities for scars and controls in different body locations, by Fitzpatrick Types and gender were estimated. Box plots were used to visualize velocities across different body locations, Fitzpatrick types and gender. A bar graph was constructed to visualize the difference in velocity between each scar and its matched control.

2.7.1. *Concurrent validity*

To evaluate if increasing velocity is representative of increasing scar stiffness (reduction of scar pliability), the concurrent validity between shear wave velocity and both the POSAS and VSS pliability sub-scores were evaluated. Both pliability sub-scores have been validated as independent measures and are therefore suitable reference standards in lieu of a “gold standard” [47,48]. Spearman’s rank correlation co-efficient was evaluated between the measured mean velocity, the mean diff-velocity and both the POSAS and VSS pliability sub-scores. A Spearman’s rho between 0.9–1 (−0.9 to −1) indicate very high correlations, 0.7–0.9 (−0.7 to −0.9) high correlations, 0.5–0.7 (−0.5 to −0.7) moderate correlations and below 0.5 (above −0.5) low correlations [49]. High correlations (above 0.7 or below −0.7) are recommended to demonstrate acceptable concurrent validity [50].

2.7.2. *Multivariable analysis*

A multivariable mixed effects model with random intercepts at the matched pair (scar/control) level was constructed to estimate adjusted mean velocities for scar tissue after taking within person correlation, gender, time since burn injury, Fitzpatrick Type, age and location of scar into account. Raw velocity measures were inverse transformed to approximate a normal distribution. Inclusion of plausible interaction terms were assessed using likelihood ratio tests. Goodness of model fit was assessed by visualization of standardized residuals plotted against fitted values. The functional form of continuous independent variables was assessed using fractional polynomial transformations. Predicted mean velocities and standard errors were back transformed to the original scale. It was hypothesized that gender, body location, Fitzpatrick Type and age would demonstrate significant associations with velocity.

3. Results

A total of 32 participants were recruited, with a total of 48 scars and 48 matched contralateral control sites assessed. All 48 pairs of scars and controls were included in the analyses. A majority of participants (n = 31, 97%) presented with a minor burn (≤20% Total Body Surface Area), and 71% (n = 34) of the scars were classified as HTS. Table 2 summarizes the details of the participants and scars.

Table 2 – Demographics of participants.

Patient details	Total
Participants	32
Gender: male/female	18/14 (56/44%)
Months after injury: mean (range)	10 (1–39)
Age: mean, SD (range)	42, 15 (19–73)
%TBSA: mean (range)	5% (0.25%–25%)
Mechanism of injury	
Flame	10 (31%)
Scald	13 (41%)
Contact	4 (12.5%)
steam	0
Friction	1 (3%)
Chemical	4 (12.5%)
Fitzpatrick type:	
I Ivory White	0
II White	12 (37%)
II Light Tan	9 (28%)
IV Moderate Brown	3 (9%)
V Dark Brown	6 (19%)
VI Brown/Black	2 (6%)
Scar details	
Scar and control pairs	48
Hypertrophic Scars = VSS height >0	34 (71%)
Scar location: n (%)	
Upper arm	4 (8%)
Lower arm	8 (17%)
Wrist/hand	4 (8%)
Thigh	12 (25%)
Calf	5 (10%)
Shin	2 (4%)
Ankle/foot	9 (19%)
Chest	1 (2%)
Back	1 (2%)
Abdomen	2 (4%)
VSS pliability	
0: normal	7 (15%)
1: subtle	13 (27%)
2: yielding	8 (17%)
3: firm	20 (42%)
4: banding	0
5: contracture	0
VSS Height	
0: normal	14 (29%)
1: <0–1 mm	16 (33%)
2: >1–2 mm	11 (23%)
3: >2–4 mm	4 (8%)
4: >4 mm	3 (6%)
POSAS pliability: mean (SD) range	3.87 (2.46) 0–8
POSAS thickness: mean (SD) range	3.17 (1.94) 0–8
POSAS relief: mean (SD) range	2.42 (2.03) 0–8

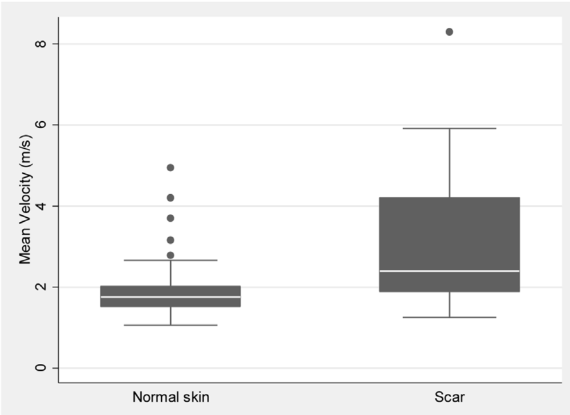

Fig. 2 – Box plot of the median velocity of scars and control skin.

The measured mean velocity of scars was 3.06 m/s, SD = 1.53 m/s, and controls was 1.93 m/s SD = 0.75 m/s. Boxplots of scar and control velocities are presented in Fig. 2. The one scar outlier was located on the dorsal hand and was confirmed to be a severe scar. Outliers in controls were located on either the shin, calf, ankle/foot, or back (Fig. 3). Table 3 summarizes the mean and standard deviation of the measured velocity and converted shear modulus of scars and matched controls by body location, gender, and Fitzpatrick type.

The mean difference in velocity between scars and matched controls was 1.15 m/s, SD = 1.51 m/s (range −0.9 m/s to 6.43 m/s). Ten scars (21%) had velocity values below the matched control. A bar graph was constructed to show the difference in velocity between each scar and its matched control (Fig. 4).

3.0.1. *Concurrent validity of SWE*

Spearman's rho indicated high correlations between measured scar velocity and POSAS pliability (r = 0.74), and between measured scar velocity and VSS pliability (r = 0.73). The diff-velocity demonstrated moderate correlations with POSAS pliability (r = 0.66), and VSS pliability (r = 0.63). Additional correlations are presented in Table 4. Of note, POSAS thickness demonstrated high correlations with measured velocity (r = 0.70), and moderate correlations with diff-velocity (r = 0.62). High correlations were also demonstrated between the VSS and POSAS pliability subscales (r = 0.91).

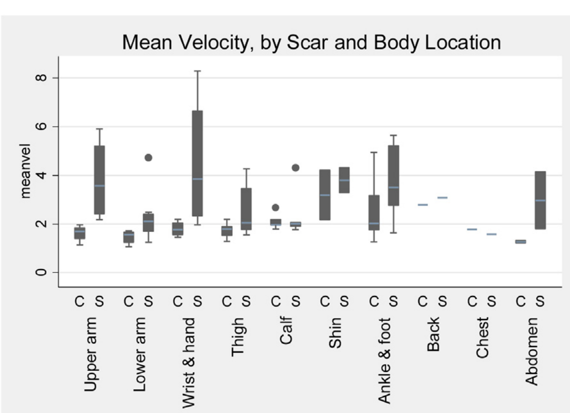

Fig. 3 – Box plot illustrating the median velocity of scars and controls by body location.

3.0.2. *Multivariable analysis*

The multivariable mixed effects linear regression, which accounted for the clustering of multiple matched sites on individuals, suggested that scars were on average 0.76 m/s stiffer than controls (CI 0.47 1.05, $p < 0.001$). The final model included significant interactions between scars, matched controls, gender and time-since-burn injury. These interactions are not represented by a single value and are therefore represented graphically (Fig. 5). The graph shows how the predicted mean shear-wave velocities associated with scars decrease over time since burn injury in both males and females. Males and females had similar mean velocities associated with scars however females had lower mean velocities at control sites compared to males. In other words, females had relatively stiffer scars compared to men when comparing scars to matched controls. There also appears to be more variation in female scar velocity measures as seen by the wide confidence intervals, but this needs to be confirmed in a larger study.

Body location and Fitzpatrick type were significantly associated with velocity in the same mixed model represented in Fig. 5. There was a tendency for the mean velocities in lower leg to be higher than mean velocities taken from the thigh. However, there was no difference between other body site locations and the thighs observed. There was some evidence that patients with Fitzpatrick type IV tended to have lower mean velocities compared to patients with Fitzpatrick type II (Table 5). Age did not demonstrate a significant association with velocity and was therefore excluded from the model.

Normal skin varied significantly by gender and by body location. Male skin was significantly stiffer than female skin ($p < 0.001$). Skin on the thighs (p = 0.006), calves ($p < 0.001$), shins ($p < 0.001$), ankles/feet ($p < 0.001$) and back (p = 0.002) were significantly stiffer than the upper arm. Neither the Fitzpatrick Type or age demonstrated significant associations with skin stiffness.

4. Discussion

This study provided initial evidence that measured velocity using SWE has concurrent validity with both the POSAS and VSS pliability sub-scores. High (>0.7) Spearman's correlations indicate that SWE is providing measurements of scar stiffness that align with the clinical presentation of pliability. The diff-velocity demonstrated moderate correlations of 0.66 and 0.63 with POSAS pliability and VSS pliability respectively, and therefore did not reach the threshold for concurrent validity. However, as demonstrated in both this and prior studies, the pliability of normal skin varies by gender, body site and age. Clinicians may not have the tactile sensitivity to discriminate the variations in normal skin pliability and

Table 3 – Velocity and shear modulus measurements for scars and control sites, by gender, body location and Fitzpatrick skin type.

	Number of paired scar-controls	Mean velocity(m/s)		Mean shear modulus (kPa)	
		Control (M, SD)	Scar (M, SD)	Control (M, SD)	Scar (M, SD)
Overall	48	1.93, 0.75	3.06, 1.53	13.03, 12.81	36.39, 39.37
Hypertrophic scars	34		3.40 m/s, 1.61		43.95, 43.53
Male	18	2.12, 0.85	3.18, 1.66	15.62, 15.62	38.54, 46.99
Female	14	1.66, 0.55	3.06, 1.85	9.22, 6.56	38.41, 54.69
Upper arm	4	1.62, 0.56	3.81, 1.62	8.82, 6.68	51.21, 42.06
Lower arm	8	1.47, 0.26	2.30, 1.05	6.67, 2.32	19.15, 20.52
Wrist/hand	4	1.79, 0.33	4.49, 2.80	10.01, 3.75	83.51, 94.88
Thigh	12	1.73, 0.31	2.52, 1.06	9.25, 3.37	22.37, 19.88
Calf	5	2.09, 0.38	2.34, 1.06	13.50, 5.30	19.75, 23.31
Shin	2	3.19, 1.09	3.80, 0.71	33.87, 21.12	44.72, 16.72
Ankle/foot	9	2.42, 1.15	3.79, 1.75	21.55, 21.42	51.82, 46.83
Back	1	2.78, 0.29	3.08, 0.42	23.48, 4.88	29.0, 8.20
Chest	1	1.77, 0.27	1.58, 0.08	9.63, 2.83	7.51, 0.77
Abdomen	2	1.26, 0.11	2.96, 1.35	4.83, 0.83	31.57, 25.10
Fitzpatrick II	12	2.01, 0.89	3.30, 1.85	14.53, 16.17	43.01, 56.60
Fitzpatrick III	9	1.90, 0.74	3.31, 1.68	12.44, 10.72	41.14, 42.30
Fitzpatrick IV	3	2.19, 0.96	2.10, 6.02	17.08, 17.00	14.31, 9.15
Fitzpatrick V	6	1.84, 0.46	2.87, 1.90	10.76, 5.95	35.40, 60.97
Fitzpatrick VI	2	1.45, 0.22	3.64, 1.48	6.45, 1.96	46.11, 35.11

m/s = meters per second, kPa = kilopascal, M = mean, SD = standard deviation.

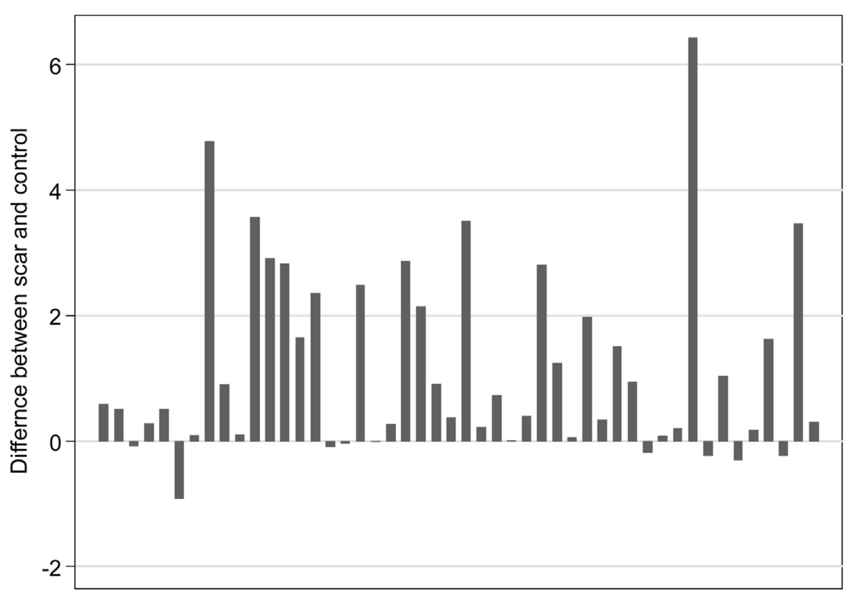

Fig. 4 – Bar graph showing the calculated difference in velocity between scar and control for each participant.

therefore, may be less accurate than the objective tool in evaluating the relative differences between scar and matched control. In the absence of a gold standard, concurrent validity only demonstrates that two assessment tools are measuring a similar criterion, it does not indicate that one tool is superior to another [48]. Further studies to evaluate the validity of diff-velocity is suggested, and in the absence of a gold standard for burn scar assessment, it is recommended to evaluate if SWE can predict clinically relevant outcomes [48].

Recent studies also evaluating correlations between the POSAS, VSS and SWE in pathological scarring have provided mixed results. Guo [51] evaluated correlations between the mean velocity of 139 keloids and the total VSS scores. The keloids had a range of total scores between 5–15 with a median

Table 4 – Spearman's correlation coefficients.

	Measured Velocity	Calculated difference in velocity	VSS height	VSS pliability	POSAS relief	POSAS pliability	POSAS thickness
Measured velocity	1.0						
Calculated difference in velocity	0.81*	1.0					
VSS height	0.51*	0.45*	1.0				
VSS pliability	0.73*	0.63*	0.69*	1.0			
POSAS relief	0.52*	0.49*	0.86*	0.72*	1.0		
POSAS pliability	0.74*	0.66*	0.61*	0.91*	0.70*	1.0	
POSAS thickness	0.70*	0.62*	0.52*	0.79*	0.63*	0.87*	1.0

All 48 scars were included in this analysis.
* Significant at <0.05.

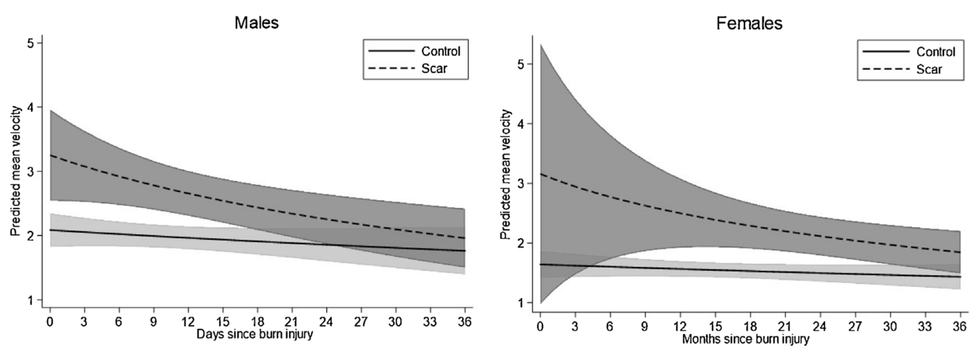

Fig. 5 – Predicted mean velocities of scar and control sites by gender and time after burn.

Table 5 – Factors associated with velocities as estimated from the same multivariable mixed effects linear regression depicted in Fig. 5.

	Predicted mean velocity difference	95% lower confidence limit	95% upper confidence limit	p-Value
Location				
Upper arm	0.18	−0.24	0.59	0.407
Lower arm	−0.18	−0.45	0.09	0.183
Wrist & hand	0.19	−0.30	0.67	0.451
Thigh	reference			
Calf	0.57	0.01	1.12	0.046
Shin	1.36	−0.11	2.83	0.070
Ankle & foot	0.61	0.18	1.04	0.005
Back	−3.58	−1903.62	1896.47	0.997
Chest	−0.40	−0.85	0.06	0.086
Abdomen	−0.31	−0.69	0.07	0.109
Fitzpatrick				
II	reference			
III	−0.24	−0.61	0.12	0.192
IV	−0.55	−0.93	−0.16	0.005
V	−0.25	−0.71	0.22	0.297
VI	0.47	−0.48	1.42	0.336

All 48 scars and matched controls were included in the analysis.
The model also included an interaction term between gender and time since burn injury and because time is a continuous variable, the results are presented graphically (Fig. 5).

of 9, and had high Spearman's rho of 0.904 ($p < 0.001$). On the other hand, Zuccaro [52] evaluated correlations between SWE and all the sub-scores (personal communication 10/02/2020) from both the VSS and POSAS in pediatric burn HTSs (n = 16) but did not find any significant correlations. The low number of participants in Zuccaro's study, and different methodologies between the studies may explain the different results. Zuccaro used an ACUSON S2000 (Siemens) with a Linear probe

operating at 9 MHz with a thin layer of ultrasound gel (standard imaging protocol), without providing evidence of reliability. We applied the same protocol in a previous study and found inconsistent consecutive images [22], leading to the development of the 10 mm thick custom-made stand-off for use with the 9 MHz probe. Reliability of this protocol was excellent [22], and was used in the current study. On the other hand, Guo used the Aixplorer US system (Supersonic Imagine, Aix-en-Provence, France) with a linear probe operating at 4–15 MHz also with a thin layer of gel, and had excellent reliability (ICC > 0.9). Different frequency probes require different protocols to maintain the skin within the focal zone. The higher frequency probe is better suited for imaging superficial tissues and therefore did not require a thick stand-off to obtain reliability. Furthermore, similar to our study, Guo used shear-wave velocity measurements in their study, whereas Zuccaro used shear modulus. The algorithms for converting shear-wave velocity into shear modulus haven't been fully tested for accuracy in superficial tissues so the use of shear moduli may also contribute to the lack of correlation between SWE and subjective scar scales in their study [17]. SWE is in its infancy for the use in dermatological conditions, further research is required to further develop optimal protocols for different frequency probes.

In addition to high correlations between velocity and pliability sub-scores, high correlations were also demonstrated between velocity and POSAS scar thickness (r = 0.70). Significant associations between scar stiffness and thickness have previously been found [43,53] and a relationship between skin thickness and stiffness is well documented [17,54–56]. A limitation of our study was the inability to use B-mode ultrasound to quantitatively measure skin/scar thickness. As mentioned, the SWE probe used in this study operates at 9 MHz frequency which is below the recommended minimum 15 MHz for skin thickness evaluation [57]. Studies combining the use of high frequency ultrasound with elastography are recommended to further investigate the relationship between skin/scar thickness and stiffness.

In addition to supporting the use of SWE to measure scar stiffness, the high Spearman's correlations between velocity, POSAS pliability and VSS pliability sub-scores supports previous studies suggesting that the pliability sub-scores can be used independently.

The hypothesis that within-patient factors are associated with velocity was demonstrated with the multivariate mixed regression analysis. Although our sample size was small, significant interactions between scars, matched controls, gender and time were observed. Overall men demonstrated higher measured velocities of normal skin compared to females, which is supported by prior research demonstrating men have stiffer skin compared to women [24,58–61]. Scar velocities in both men and women were lower with increasing time after injury, which is also consistent with previous studies suggesting scars in both genders improve with time [61]. Although, no significant differences were found between the measured velocities of male and female scars, females had relatively stiffer scars when compared to matched controls. This finding is supported by previous studies that showed females were at higher risk of developing pathological scars compared to men [62,63].

Further associations between velocity, body location and Fitzpatrick Type were found in the regression after adjusting for the interaction between scars, matched controls, gender and time. Unexpectedly, Fitzpatrick Type IV scars had significantly lower velocities compared to both matched controls and to Fitzpatrick type II scars. Fitzpatrick Type IV includes Asian skin types, which are known to develop aggressive scars [63–65]. The low number of patients in the Type IV subgroup may explain this finding. However, the velocity of Type IV controls fits within the range of a study conducted on healthy individuals in China (range = 1.41–3.13 m/s, mean = 2.53 m/s) using the same elastography system as our study [66]. Another possible explanation of the low velocities for Type IV scars is that the results of our study did not account for clinical interventions; therefore, it is plausible that these were aggressive scars that have undergone therapies reducing their stiffness to below that of the matched controls. Further studies investigating the effects of various interventions, and studies powered to investigate the association between velocity and Fitzpatrick skin types are recommended.

Age was not associated with either skin or scar velocity in this small study which is inconsistent with previous research [25–27,60,67]. Age was treated as a continuous variable in our study, whereas other studies have used various age categories when evaluating their data. Being an exploratory study, the sample size was not powered to account for the numerous variables that may influence velocity and is a potential reason for the wide confidence intervals noted in this study. Therefore, age should not be discounted until further studies on larger sample sizes are conducted.

The significant interactions and associations found in the regression of such a small sample suggests that within-patient factors can potentially bias the results of future studies and therefore need to be considered when interpreting velocity values. Furthermore, the use of a single cut-off value to discriminate pathological scars, as suggested by our previous study, is not recommended. The mean measured velocity of normal skin on the back, shin, and ankle/foot were higher than the pathological scar cut-off value suggested in our previous study of 2.22 m/s. Previous burn studies have also suggested various cut-off values to define pathological scars using either subjective or objective scales [44,68], however they too did not account for within-patient factors. This suggests that the diff-velocity is still worth pursuing as a measurement protocol, even though it did not reach the threshold for concurrent validity in this study. Objective assessment tools have the potential to provide physiological biomarkers of pathological scarring which can be used to predict long term scar outcome and evaluate the effectiveness of treatments that target scar physiology. The diff-velocity allows comparison of the scar to a 'baseline' of normal comparative skin, therefore, the use of ratio scores between the scar and control may be worth developing in future studies. However not every scar will have a matched control, therefore the measured velocity is valuable for research measuring change in the scar over time, and for clinical scar assessment. Clinical assessment has a greater emphasis on evaluating treatment efficacy for each individual and SWE has the potential to provide point-of-care assessment so that personalized approaches to treatment can be

implemented. The measured velocity is also less time consuming than diff-velocity as control sites don't need to be measured, which is important in clinical practice.

A novel finding in our research was that 20% of scars had velocities below that of the matched control. This is in contrast to previous studies that have suggested scars do not return to normal levels of pliability [61]. As mentioned, interventions used to prevent or minimize scars were not accounted for in this study which may explain this finding. Longitudinal studies are recommended to further investigate this finding.

A few limitations need to be considered when interpreting our study. Firstly, due to a lack of a gold standard for evaluating scar biomechanical properties, subjective clinical assessments were used which may influence the correlation results. In addition, due to the small sample size, this study did not have any scars scoring above 3 in the VSS pliability sub-score which may have influenced both correlation and regression analysis. Furthermore, numerous life-style (e.g. smoking, alcohol consumption, BMI), environmental (e.g. sun exposure, pollutant exposure) and burn related factors (e.g. TBSA, burn depth, treatment) will influence healing capacity, scar formation, and therefore velocity. These factors are important to consider and may have contributed to the wide confidence intervals. The aim of this study however, was to explore if variables that are unable to be manipulated, such as age, gender and skin type influence the interpretation of the measurements and the definition of scar stiffness severity. This is essential to ensure further studies using SWE can accurately evaluate the impact of life-style, environmental and burn related factors on tissue healing. Larger, longitudinal studies powered to evaluate the interactions and associations between all appropriate variables can then be developed in the future to gain a better understanding of which factors can be manipulated to improve tissue healing, scarring and patient outcome.

5. Conclusion

This study has provided initial evidence that SWE can quantify adult burn scars, using the measured velocity of scar stiffness. Significant associations in the regression analysis suggest that scar velocity varies by gender, body location and Fitzpatrick skin type. These factors, and the time of assessment following the injury need to be considered when defining values of scar severity using velocity. SWE shows potential to be a valuable tool to evaluate scar stiffness in both clinical and research settings. Further research is recommended on larger samples, and in combination with high-frequency ultrasound to further expand on these results.

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Conflict of interests

Siemens Heathineers provided the S3000 system for the use of this research. However, the company was not involved in the design or conduction of this research, nor did they have the opportunity to edit or review this paper prior to publication.

Brendan Kennedy is a founder and shareholder in OncoRes Medical (Perth, Western Australia, Australia), a start-up company developing optical-based elastography for surgical applications. In addition, he performs funded research for this company.

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REFERENCES

[1] Barnes LA, et al. Mechanical forces in cutaneous wound healing: emerging therapies to minimize scar formation. Adv Wound Care 2018;7(2):47–56.
[2] Harn HIC, et al. The tension biology of wound healing. Exp Dermatol 2019;28(4):464–71.
[3] Fernandes M, da Silva L, Marques A. Skin mechanobiology and biomechanics: from homeostasis to wound healing. Advances in Biomechanics and Tissue Regeneration. Elsevier Science Pub. Co.; 2019. p. 343–60.
[4] Sullivan T, et al. Rating the burn scar. J Burn Care Rehabil 1990;11(3):256–60.
[5] Forbes-Duchart L, et al. Burn therapists' opinion on the application and essential characteristics of a burn scar outcome measure. J Burn Care Res 2009;30(5):792–800.
[6] McOwan CG, MacDermid JC, Wilton J. Outcome measures for evaluation of scar: a literature review. J Hand Ther 2001;14 (2):77–85.
[7] Fearmonti R, et al. A review of scar scales and scar measuring devices. Eplasty 2010;10:e43.
[8] Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 2014;15(12):802–12.
[9] Busche MN, et al. Burn scar evaluation using the cutometer® MPA 580 in comparison to "Patient and Observer Scar Assessment Scale" and "Vancouver Scar Scale". J Burn Care Res 2017;39(4):516–26.
[10] Nedelec B, et al. Quantitative measurement of hypertrophic scar: interrater reliability and concurrent validity. J Burn Care Res 2008;29(3):501–11.
[11] Goei H, et al. Predictive validity of short term scar quality on final burn scar outcome using the Patient and Observer Scar Assessment Scale in patients with minor to moderate burn severity. Burns 2017;43(4):715–23.
[12] Lee KC, et al. A systematic review of objective burn scar measurements. Burns Trauma 2016;4(1):14.
[13] Baxter SC. An introductory guide to solid mechanics. Mechanobiologie Handbook. CRC Press, Taylor & Francis Group; 2011. p. 3–44.
[14] Draaijers LJ, et al. Skin elasticity meter or subjective evaluation in scars: a reliability assessment. Burns 2004;30 (2):109–14.
[15] Nedelec B, et al. Quantitative measurement of hypertrophic scar: intrarater reliability, sensitivity, and specificity. J Burn Care Res 2008;29(3):489–500.

[16] Gankande TU, et al. Reliability of scar assessments performed with an integrated skin testing device–the DermaLab Combo. Burns 2014.
[17] Lee SY, et al. Preliminary results on the feasibility of using ARFI/SWEI to assess cutaneous sclerotic diseases. Ultrasound Med Biol 2015;41(11):2806–19.
[18] Dietrich C, et al. EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017 (Long Version). 2017.
[19] Ferraioli G, et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 3: liver. Ultrasound Med Biol 2015;41(5):1161–79.
[20] Barr RG, et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: part 2: breast. Ultrasound Med Biol 2015;41(5):1148–60.
[21] Creze M, et al. Shear wave sonoelastography of skeletal muscle: basic principles, biomechanical concepts, clinical applications, and future perspectives. Skeletal Radiol 2018;1–15.
[22] DeJong HM, et al. A novel, reliable proocol to objectively assess scar stifness using shear-wave elastography (in Press). Ultrasound Med Biol 2020.
[23] Nedelec B, et al. Skin characteristics: normative data for elasticity, erythema, melanin, and thickness at 16 different anatomical locations. Ski Res Technol 2016;22(3):263–75.
[24] Xiang X, et al. Quantitative assessment of healthy skin elasticity: reliability and feasibility of shear wave elastography. Ultrasound Med Biol 2017;43(2):445–52.
[25] Pawlaczyk M, Lelonkiewicz M, Wieczorowski M. Age-dependent biomechanical properties of the skin. Adv Dermatol Allergol 2013;30(5):302.
[26] Dulińska-Molak I, et al. Age-related changes in the mechanical properties of human fibroblasts and its prospective reversal after anti-wrinkle tripeptide treatment. Int J Pept Res Ther 2014;20(1):77–85.
[27] Luebberding S, Krueger N, Kerscher M. Mechanical properties of human skin in vivo: a comparative evaluation in 300 men and women. Ski Res Technol 2014;20(2):127–35.
[28] Pham H, Reece B, Hines M. Loss of skin elasticity is more dependent on Fitzpatrick skin type than chronologic age. J Am Acad Dermatol 201572(5).
[29] Lipman SL, et al. Evaluating the improvement in shear wave speed image quality using multidimensional directional filters in the presence of reflection artifacts. IEEE Trans Ultrason Ferroelectr Freq Control 2016;63(8):1049–63.
[30] Bamber J, et al. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: basic principles and technology. Ultraschall in der Medizin-European J Ultrasound 2013;34(02):169–84.
[31] Thompson CM, et al. What score on the Vancouver Scar Scale constitutes a hypertrophic scar? Results from a survey of North American burn-care providers. Burns 2015;41(7):1442–8.
[32] Gankande T, et al. A modified Vancouver Scar Scale linked with TBSA (mVSS-TBSA): inter-rater reliability of an innovative burn scar assessment method. Burns 2013;39(6):1142–9.
[33] Baryza M, Baryza G. The Vancouver Scar Scale: an administration tool and its interrater reliability. J Burn Care Rehabil 1995;16(5):535–8.
[34] van der Wal MB, et al. Differential item functioning in the Observer Scale of the POSAS for different scar types. Qual Life Res 2014;23(7):2037–45.
[35] Nedelec B, Shankowsky H, Tredget E. Rating the resolving hypertrophic scar: comparison of the Vancouver Scar Scale and scar volume. J Burn Care Rehabil 2000;21(3):205–12.
[36] Draaijers LJ, et al. The patient and observer scar assessment scale: a reliable and feasible tool for scar evaluation. Plast Reconstr Surg 2004;113(7):1960–5.
[37] Nicholas RS, et al. Patient-related keloid scar assessment and outcome measures. Plast Reconstr Surg 2012;129(3):648–56.
[38] Draaijers LJ, et al. Colour evaluation in scars: tristimulus colorimeter, narrow-band simple reflectance meter or subjective evaluation? Burns 2004;30(2):103–7.
[39] Lau JC, Li-Tsang CW, Zheng Y. Application of tissue ultrasound palpation system (TUPS) in objective scar evaluation. Burns 2005;31(4):445–52.
[40] Lye I, et al. Tissue tonometry is a simple, objective measure for pliability of burn scar: is it reliable? J Burn Care Res 2006;27 (1):82–5.
[41] Candy LHY, Cecilia L-TWP, Ping ZY. Effect of different pressure magnitudes on hypertrophic scar in a Chinese population. Burns 2010;36(8):1234–41.
[42] Li-Tsang CW, Lau JC, Chan CC. Prevalence of hypertrophic scar formation and its characteristics among the Chinese population. Burns 2005;31(5):610–6.
[43] Lee K, et al. Burns objective scar scale (BOSS): validation of an objective measurement devices based burn scar scale panel. Burns 2019.
[44] van der Wal MB, et al. Rasch analysis of the Patient and Observer Scar Assessment Scale (POSAS) in burn scars. Qual Life Res 2012;21(1):13–23.
[45] van de Kar AL, et al. Reliable and feasible evaluation of linear scars by the Patient and Observer Scar Assessment Scale. Plast Reconstr Surg 2005;116(2):514–22.
[46] Draaijers LJ, et al. Skin elasticity meter or subjective evaluation in scars: a reliability assessment. Burns 2004;30(2):109–14.
[47] Brink Y, Louw QA. Clinical instruments: reliability and validity critical appraisal. J Eval Clin Pract 2012;18(6):1126–32.
[48] Trikalinos TA, Balion CM. Options for summarizing medical test performance in the absence of a “gold standard”. J Gen Intern Med 2012;27(1):67–75.
[49] Mukaka MM. A guide to appropriate use of correlation coefficient in medical research. Malawi Med J 2012;24 (3):69–71.
[50] Terwee C, et al. Quality criteria were proposed for measurement properties of health status questionnaires. J Clin Epidemiol 2007;60(1):34–42.
[51] Guo R, et al. Quantitative Assessment of Keloids using ultrasound shear wave elastography. Ultrasound Med Biol 2020.
[52] Zuccaro J, et al. Quantification of pediatric burn scar stiffness using acoustic radiation force impulse ultrasound elastography. Ultrasound Med Biol 2019.
[53] Simons M, et al. Ultrasound is a reproducible and valid tool for measuring scar height in children with burn scars: a cross-sectional study of the psychometric properties and utility of the ultrasound and 3D camera. Burns 2017;43 (5):993–1001.
[54] Smalls LK, Randall Wickett R, Visscher MO. Effect of dermal thickness, tissue composition, and body site on skin biomechanical properties. Ski Res Technol 2006;12(1):43–9.
[55] Yang Y, et al. Quantitative assessment of skin stiffness using ultrasound shear wave elastography in systemic sclerosis. Ultrasound Med Biol 2019.
[56] Wang L, et al. Quantitative assessment of skin stiffness in localized scleroderma using ultrasound shear-wave elastography. Ultrasound Med Biol 2017;43(7):1339–47.
[57] Wortsman X, et al. Guidelines for performing dermatologic ultrasound examinations by the DERMUS group. J Ultrasound Med 2016;35(3):577–80.
[58] Ambroziak, M., et al., Ultrasonographic elastography in the evaluation of normal and pathological skin–a review.
[59] Sun Y, et al. Reproducibility analysis on shear wave elastography (SWE)-based quantitative assessment for skin elasticity. Medicine 201796(19).
[60] Yang Y, et al. Determination of normal skin elasticity by using real-time shear wave elastography. J Ultrasound Med 2018.
[61] Nedelec B, et al. Longitudinal burn scar quantification. Burns 2014;40(8):1504–12.

[62] Gangemi EN, et al. Epidemiology and risk factors for pathologic scarring after burn wounds. Arch Facial Plast Surg 2008;10(2):93–102.
[63] Wallace HJ, et al. Identification of factors predicting scar outcome after burn in adults: a prospective case–control study. Burns 2017;43(6):1271–83.
[64] Sood RF, et al. Race and melanocortin 1 receptor polymorphism R163Q are associated with post-burn hypertrophic scarring: a prospective cohort study. J Invest Dermatol 2015;135(10):2394–401.
[65] Schulze C, et al. Stiffening of human skin fibroblasts with age. Biophys J 2010;99(8):2434–42.
[66] Wang Y, et al. Comparison of 2-D shear wave elastography with clinical score in localized scleroderma: a new method to increase the diagnostic accuracy. J Dermatol 2018.
[67] Diridollou S, et al. Skin ageing: changes of physical properties of human skin in vivo. Int J Cosmet Sci 2001;23(6):353–62.
[68] Oliveira GV, et al. Objective assessment of burn scar vascularity, erythema, pliability, thickness, and planimetry. Dermatol Surg 2005;31(1):48–58.