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Evaluation of Ultrasound Changes With the Use of Microneedling Versus Fractional CO2 Laser in Atrophic Acne Scars

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ABSTRACT

Introduction: Atrophic acne scarring, a common sequela of acne, can be treated by different interventions, including microneedling and laser resurfacing.

Objectives: We sought to evaluate the comparative efficacy of microneedling versus fractional CO2 laser in treating atrophic acne facial scars using imaging with high and ultra-high frequency ultrasound.

Methods: Participants received 2 sessions, separated by 1 month, of microneedling on the left side of the face and fractional CO2 laser on the right. Color Doppler ultrasound evaluations (24 and 70 MHz) were conducted at baseline and 3 months after treatment. Each patient completed questionnaires on satisfaction, pain, and adverse effects.

Results: Nine subjects were enrolled. The frequency order of scar types was boxcar, ice-pick, and rolling. At 3 months, using the acne scar clinical evaluation scale, a decrease in scar scores of both methods was observed for total scars (P = 0.0005), ice-pick scars (P = 0.0128), and rolling scars (P = 0.0007). Twenty-two scars analyzed by ultrasound demonstrated a trend to decrease in size; however, no significant changes were observed for either microneedling or CO_2 laser treatments. Moreover, there were no significant differences between these methods. Both treatments were rated as good or very good by patient assessments. There was a low frequency of pain and hyperpigmentation reported with both modalities, albeit somewhat higher with microneedling.

Conclusions: Both microneedling and CO_2 laser improved atrophic acne scars. Ultrasound did not show significant differences between these modalities.

Introduction

Acne vulgaris is a chronic inflammatory dermatosis of the pilosebaceous unit characterized by comedones, papules, pustules, and/or nodules [1]. One of the main sequelae is scarring, which can be present in up to 95% of cases, with no difference between genders [2]. Post acne scars develop from dermal matrix destruction with inadequate matrix repair subsequent to inflammation of primary lesions and self-manipulation [3-5].

In 2001, Jacob et al distinguished 3 main subtypes of atrophic scars based on morphological characteristics of width, shape, and depth [6]. The ice-pick type (in a "V" or pitted-ice pattern) has a wide opening with a deep extension narrowing into the dermis, with a diameter of less than 2-3 mm. The rolling type (in a "U" or circular pattern) is wide, 4-5 mm in diameter, with variable depth and a wavy appearance. The boxcar type (in an "M" or rectangular or saucer shape) has a diameter of 1.5 to 4 mm, without narrowing as it extends into the dermis [5,7,8]. Their prevalence is estimated to be 60%-70%, 20%-30%, and 15%-25% of patients, respectively [6]. These can also be classified according to depth as superficial (0.1 to 0.5 mm) or deep (greater than 0.5 mm) [9].

While scar correction procedures are imperfect, some modalities have been considered more effective for certain atrophic scar morphologies [3,10]. Both microneedling and fractional CO_2 laser have been reported to be efficacious [10-13].

Objectives

This study aims to evaluate the ultrasonographic changes, adverse effects, and patient satisfaction following treatment with microneedling versus fractional CO_2 laser in patients with atrophic acne scars. We hypothesized that both methods would exhibit similar changes 3 months after the last treatment.

Methods

Study Population

A non-randomized 16-week prospective study was conducted by the Departments of Dermatology of Universidad de los Andes and Clinica Davila, as well as the Institute for Diagnostic Imaging and Research of the Skin and Soft Tissues in Santiago, Chile.

Inclusion criteria were men and women >18 years, with skin phototypes III-IV, with a clinical diagnosis of superficial rolling and/or boxcar atrophic scars located on the face. Screening of subjects was undertaken from April to May 2021.

Exclusion criteria were pregnancy, lactation, use of isotretinoin, laser and/or surgical procedures on the face within the preceding six months, tobacco use in the prior six weeks, active infection at the time of evaluation, personal or family history of keloid or hypertrophic scarring, bleeding disorders, photosensitivity, connective tissue disease, allergy to topical and/or local anesthesia, and medications associated with hyperpigmentation.

This protocol was approved by Clinica Davila ethics committee (#05042021), which also includes Department of Dermatology of Universidad de los Andes and Institute for Diagnostic Imaging and Research of the Skin and Soft Tissues. All patients provided informed consent to participate in the study and for publication of their images. All procedures were carried out under the principles of the Helsinki Declaration.

Study Procedure

Two weeks before each session, facial creams were discontinued, and adapalene 0.1% gel was prescribed for nightly use on both cheeks. On the day of the procedure, 2.5% lidocaine + 2.5% prilocaine anesthetic cream was applied to both cheeks for 30 minutes and then removed with micellar water. The area to be treated was delimited along its horizontal axis (area between the outer corner of the eye and the tragus) and its vertical axis (lip corner to the angle of the jaw).

The left side of the face was treated with microneedling (dermaroller, RoHS®) with a 2 mm needle length. Its use was multidirectional (vertical, horizontal, left oblique, and right oblique axis), with 10 inclinations for each direction and 5 reciprocal movements for each one. The right side of the face was treated with a fractional CO2 laser (10,600 nm, eCO2™, Lutronic®), using the following parameters: static mode, 120 mm tip, 20 W power, energy pulse of 20 mJ/msec, the density of 1.1% (100 dots/cm2). Only one pass was performed in each session without overlap. Two sessions were performed, each separated by 1 month. At the end of the procedure, a cold compress with saline solution was applied to each treated area. A base ointment was applied for the first 24 hours, which was later replaced by emollients and SPF 50 sunscreen. Topical and/or oral antibiotics were not prescribed. All patients received prophylactic treatment with valacyclovir 500 mg, one tablet every 12 hours for 3 days, regardless of a history of orolabial herpes. Patients were advised to avoid using exfoliants and sun exposure for 1 month after each session.

Assessments

Photographic, greyscale, and color Doppler ultrasound documentation were performed before and 3 months after the last treatment. Photographs were taken on each face horizontal and frontal axis, with the same camera (Nikon D3300), lens (Nikon 60mm) and lighting conditions (artificial white LED lighting).

Two ultrasound devices were used: Logic E 10 (General Electric Health Systems) with a compact linear transducer with a maximum range of 24 MHz (high-frequency) and Vevo MD (VisualSonics, Fujifilm) with a linear transducer with a maximum frequency of 70 MHz (ultra-high-frequency). The 24 MHz device recorded the type of vascular flow (arterial or venous), maximum thickness (mm), and maximum arterial peak systolic velocity (cm/sec) of the blood vessels in the scar regions. The 70 MHz device described the depth, transverse, and longitudinal diameters (mm) of the scar selected by the principal investigator.

In each patient, the largest scars were selected. In cases of similar sizes, two lesions were selected. Each scar was marked with a pen, measured with a ruler, and photographed, to enable evaluation of the same site on follow-up. Clinical changes were evaluated by two blinded dermatologists using the acne scar clinical evaluation scale (ECCA) [8]. This quasi-quantitative method considers the number and type of scars, assigning a determined numerical value that, when multiplied by its respective factor, provides a score with a minimum of 0 and a maximum of 540. The scale was applied to both cheeks simultaneously, without comparison between them.

Three months after the final treatment session, each patient completed a satisfaction survey, a visual analog scale of pain (VAS), and a questionnaire on adverse effects. The survey evaluated patient perception of improvement at each cheek after treatment, classifying it as minimal (0% to 24%), good (25% to 49%), very good (50% to 74%), and excellent (75% to 100%).

The questionnaire allowed for the subjective assessment of signs or symptoms perceived by the patients during the protocol, including pain, erythema, bleeding, pigmentation, and local infection.

Statistical Analysis

Statistical analysis was performed with RStudio (version 2022.02.2+576). After evaluating the distribution of the different variables, the Student t-test was used for those that met normality, while a non-parametric method (Wilcoxon) was used for the remainder. Statistical significance was established at $P < 0.05$.

Results

Ten subjects fulfilled inclusion and exclusion criteria and underwent treatment. However, one patient did not attend follow-up visits, leaving 9 (5 male and 4 female) available for analysis. From this cohort, 22 scars were included in ultrasonographic studies (2 participants had two scars evaluated per cheek). The mean age of this group was 30.7 years (SD +- 8,6 years; range, 19-42 years), and the skin phototypes were III (80%) and IV (20%).

Using the ECCA scale, scar types in order of frequency were boxcar, ice-pick, and rolling (Table 1). The clinical size reduction of each scar considering both sides was 3.4%, 25%, and 65.6%, with a total score of decrease of 27.3%. The variation (%) was statistically significant in ice-pick scars ($P = 0.0128$), rolling scars ($P = 0.0007$), and the total score ($P = 0.0005$).

On ultrasound (70 MHz), one patient (11.1% of total) showed reduced scar depth with microneedling, and 5 patients (55.6% of the total) demonstrated a decreased depth with laser CO2. For either transverse or longitudinal diameters, there was a trend towards reduction in all patients with microneedling and only in 7 patients (77.8% of total) with laser CO2.

On color Doppler (24 MHz), the dermal vascularity in the scar regions was predominantly arterial type with low velocity. After both types of treatment, 5 patients slightly increased the thickness of the vessels, and in 5 patients, there was a slight decrease of the blood flow velocity (peak systolic

Table 1. Acne Scar Clinical Evaluation Scale (ECCA) Before and After Both Procedures

ECCA Scale	Mean Baseline (±SD)	Mean 3-Month Follow-up (±SD)	Percentage of Variation	P-Value
Ice-Pick	40 (8.9)	30 (12.6)	–25%	0.0128
Rolling	32 (15.6)	11 (15.7)	–65.6%	0.0007
Boxcar	58 (19.1)	56 (18.3)	–3.4%	0.6187
Total Score	161 (34.9)	117 (33.5)	–27.3%	0.0005

SD = standard deviation.

Table 2. Color Doppler Ultrasound Changes at Baseline and 3 Months After Microneedling Use

Patient		Baseline					3-Month Follow-up					Percentage of Variation				
		D	V	P	T	L	D	V	P	T	L	D	V	P	T	L
1	a	-	-	0.7	1.3	3.8	0.7	2.9	1	2.4	3.8	-	-	42.9	84.6	0
	b	-	-	1.1	2.6	8	0.5	3.4	1.3	2.9	3.4	-	-	18.2	11.5	-57.5
2		0.8	8.7	0.6	0.9	0.9	0.5	3.9	1.5	0.7	0.6	-37.5	-55.1	150	-22.2	-33.3
3		0.4	8.4	1	2	1.2	0.3	-	1	1.3	2.3	-25	-	0	-35	91.6
4		0.5	4	0.9	1.8	3.3	0.5	4.5	0.9	3	1.7	0	12.5	0	66.6	-48.5
5		0.4	4.2	0.9	2.9	3	0.2	2.6	1	2.2	2.4	-50%	-38.1	11.1	-24.1	-20
6	a	0.3	4.8	0.8	1.1	1.3	0.7	5.7	1.2	3	2.7	133.3	18.8	50	172.7	107.7
	b	0.4	2.8	1.4	2.4	2.9	0.8	7.2	1.5	3	1.9	100	157.1	7.1	25	-34.5
7		0.3	9.4	1.7	5.3	4.1	0.5	4	1	1.9	1.6	66.6	-57.4	-41.2	-64.2	-61
8		-	-	1.1	1.9	3.9	0.7	10.5	1.2	1.9	1.7	-	-	9.1	0	-56.4
9		0.4	6.1	1	2.9	3.9	0.6	5.7	1	3.6	3.2	50%	-6.5	0	24.1	-17.9
		Mean (±SD)					Mean (±SD)					P-Value				
Mean		0.4 (0.2)	6.1 (2.5)	1 (0.3)	2.3 (1.2)	3.3 (2)	0.6 (0.2)	5 (2.4)	1.2 (0.2)	2.4 (0.9)	2.3 (0.9)	0.195	0.395	0.277	0.873	0.158

D = Maximum thickness of the blood vessels (mm); L = Longitudinal scar diameter (mm); P = Scar depth (mm); SD = Standard deviation; T = Transverse scar diameter (mm); V = Maximum arterial peak systolic velocity (cm/sec).

velocity of the arterial vessels), which composed 55.6% of the total for each treatment modality.

However, neither treatment caused significant changes in acne scar diameters or vascularity after ultrasound examination (Tables 2-4, Figures 1-3).

The satisfaction survey revealed that 44% of the patients had a very good or excellent perception of the results, with no significant difference between the two methods.

The visual analogue pain scale resulted in a mean score of 6.8 for microneedling, compared to 5.3 for the CO2 laser. In addition, the mean duration of the pain was 1.7 days and 1.3 days, respectively. Both procedures resulted in erythema, bleeding, and hyperpigmentation, with the first two being more prolonged with the laser while the latter was more prolonged with microneedling (Table 5). No patient had a local infection and/or orolabial herpes.

Conclusions

This study showed clinical improvement in all scar types, evidenced by the decrease in the average ECCA score of each, which was significant in ice-pick (P = 0.0128) and rolling (P = 0.0007) subtypes where the latter demonstrated a reduction of 65.6%. Moreover, the total score presented a significant decrease (P = 0.0005).

Several previous studies reported the effectiveness of microneedling and CO2 laser in acne scars treatment. In a systematic review of ablative fractional CO2 laser for facial atrophic acne scars performed in 2018 by Xu et al found that 2 to 7 treatment sessions were effective with an interval of 4 to 12 weeks, with improvement after just one session [10]. In addition, higher flow rates and lower densities have been associated with greater effectiveness [7]; however, considering that the depth of the damage depends on the energy used [11], there are no established parameters for atrophic scars, depending on the characteristics of the lesion and the individual in particular [10].

In 2020, Villani et al [12] described in a systematic review improvement with microneedling after 1-3 months of either weekly or monthly sessions [14-16]. These changes had also been proved histologically by a significant increase in collagen type I, III, and VI, and a decrease in elastin [17].

Table 3. Changes in Color Doppler Ultrasound Measurements at Baseline and After 3 Months of CO2 laser

Patient		Baseline					3-Month Follow-up					Percentage of Variation				
		D	V	P	T	L	D	V	P	T	L	D	V	P	T	L
1	a	0.4	2.8	1	3.4	4.1	0.4	7.5	1.1	3.3	1.1	0	167.9	10	-2.9	−73.2
	b	0.4	4	1.1	2.8	4.4	0.3	3.4	1.2	1.8	1.6	−25	−15	−70	−35.7	−63.6
2		0.4	5.6	0.9	2.3	1.4	0.6	3.4	1.5	1.3	1.3	50	−39.3	66.6	−43.5	−7.1
3		0.8	5.1	1.2	3	3.7	0.2	2.6	1.1	4.3	1.9	−75	−49	−8.3	43.3	−48.6
4		0.9	4.3	1.2	3.3	2.4	0.8	2.8	0.8	2.4	1.6	−11.1	−34.9	−33.3	−27.2	−33.3
5		0.2	4.2	1.2	2.4	2.5	-	-	0.8	3	1.8	-	-	−33.3	25	−30
6	a	0.4	3.4	0.4	1.4	1.7	0.7	12.5	1.5	6.9	3.5	75	267.6	275	392.9	105.9
	B	0.4	3.9	1	2.2	1.8	0.8	5.7	1.3	2.3	2.7	100	46.1	30	9.1	50
7		0.4	4.5	1.6	4.4	3.2	0.6	5.4	1.6	2.7	2.4	50	20	0	−38.6	−25
8		-	-	1.3	2.6	2.9	0.9	4.8	1.2	4.3	4.3	-	-	-7.7	69.2	48.3
9		-	-	1.3	6.3	2.3	0.5	3.5	1.4	2.7	2.5	-	-	7.7	−57.1	8.7
		Mean (+-SD)					Mean (+-SD)					P-Value				
Mean		0.5 (0.2)	4.2 (0.8)	1.1 (0.3)	3.1 (1.3)	2.8 (1)	0.6 (0.2)	5.1 (3)	1.2 (0.3)	3.2 (1.5)	2.3 (1)	0.587	0.870	0.343	1.000	0.231

D = Maximum thickness of the blood vessels (mm); L = Longitudinal scar diameter (mm); P = Scar depth (mm); SD = Standard deviation; T = Transverse scar diameter (mm); V = Maximum arterial peak systolic velocity (cm/sec).

Table 4. Ultrasonographic Mean Percentage Variation in the Blood Vessels and Scars Measurements After Both Treatments

Variable	Treatment	Mean Percentage Variation After Procedure	Standard Deviation	T-Test/ W Test	P-Value
Blood Vessels					
Maximum thickness (mm)	Microneedling	+24.44	66.08	-0.3695	0.7169
	CO2 laser	+13.28	58.51		
Maximum arterial peak systolic velocity (cm/s)	Microneedling	+4.44	74.04	36	0.3969
	CO2 laser	+46.56	113.12		
Scar Diameters (mm)					
Depth	Microneedling	+22.47	48.55	57	0.8433
	CO2 laser	+35.47	87.40		
Transverse	Microneedling	+21.74	66.31	49.5	0.4904
	CO2 laser	+29.64	126.56		
Longitudinal	Microneedling	-15.37	62.43	69.5	0.5766
	CO2 laser	+3.1	70.96		

Nabari et al in 2023 compared needling (radiofrequency microneedling, mesoneedling, microneedling) and ablative fractional lasers (CO2, erbium-YAG) in the treatment of atrophic and hypertrophic scars in a systematic review [18]. No statistically significant difference was found between the two methods and significant improvement was noted in 20% of the studies for laser and microneedling. However, none of the studies reviewed specifically compared fractional CO2 laser and microneedling in the treatment of atrophic acne scars.

Boen et al. previously described that changes with microneedling occur mainly at the level of the papillary dermis, being mainly described for rolling and shallow boxcar scars [19]. A prospective interventional study performed by Sardana et al. in 2014 with non-ablative fractional laser showed that rolling and boxcar scars respond better than ice-pick scars [20]. In our experience scars with boxcar morphology showed the smallest changes, which can be explained by the clinical difficulty in differentiating their depth in the patient selection process, given that deeper variants tend to be more

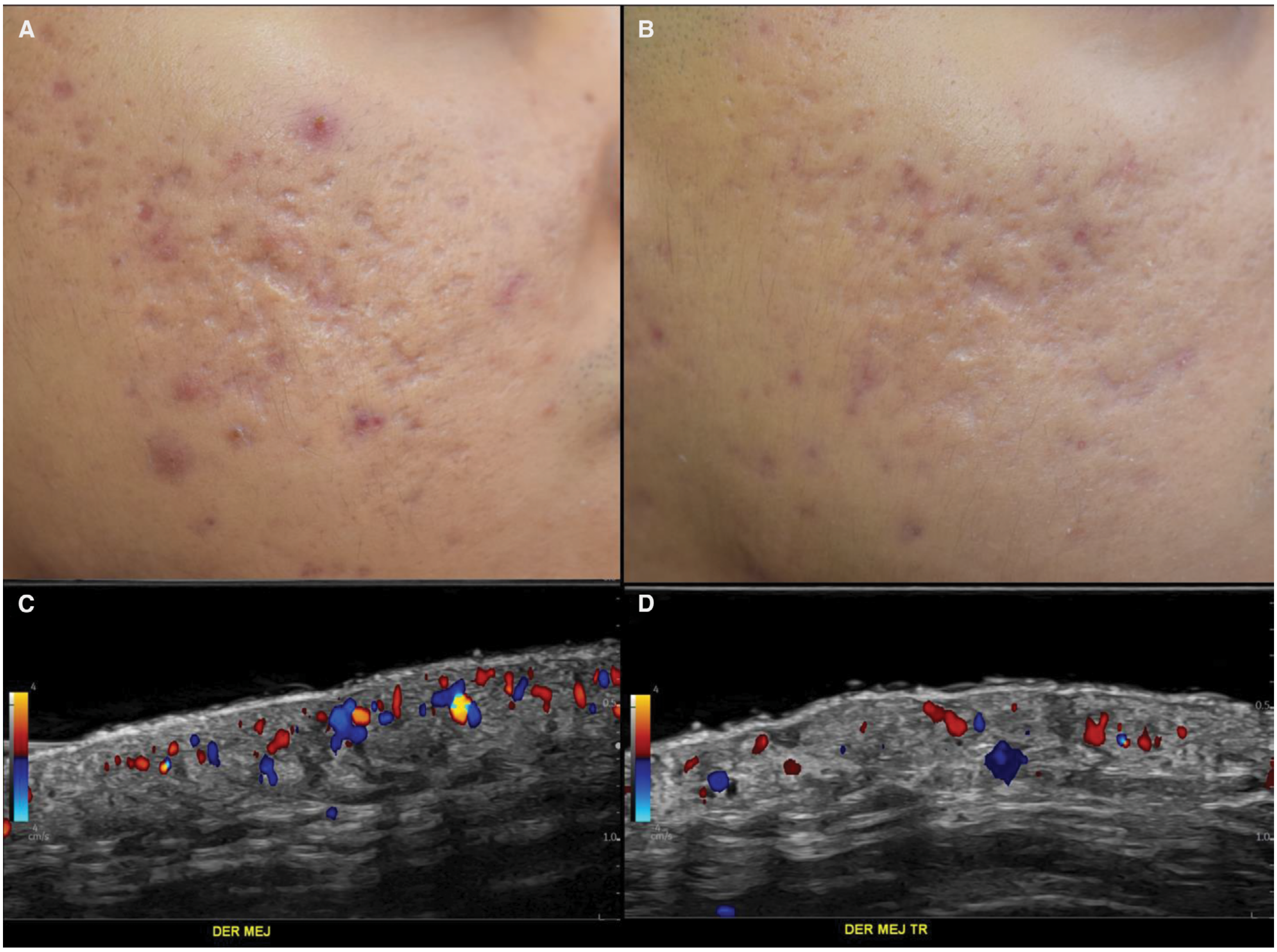

Figure 1. Effects on the right cheek with fractional CO2 laser. (A) Baseline clinical image; (B) Follow-up clinical image at 3 months. There is a decrease in erythema along with a clinical improvement in acne scars. Color Doppler high-frequency ultrasound at 24 MHz: baseline (C) and follow-up at 3 months (D). There is a decrease in dermal vascularity in the scar region (colors).

refractory to treatment. It should be noted that we did observe favorable changes in ice-pick scars.

Our study evaluated ultrasound changes after both methods. Malinowska et al. previously described statistically significant decrease in dermal thickness and scar depth using high-frequency ultrasound (48 MHz) in acne scars after 3 sessions of Er:YAG 2940 nm fractional laser [21]. We innovated using ultra-high-frequency ultrasound, which allows the evaluation of scars with a spatial axial resolution of 30 μm [22]. This device provides a higher-quality image and, therefore, a better comparison of changes in diameters over time. We found a trend of reducing longitudinal or transverse diameters with both modalities, demonstrating effectiveness with either microneedling or CO2 laser.

The vascularization in acne scars has not been previously assessed in other studies. On color Doppler, we found increased thickness of the vessels after treatment. While not statistically significant, the latter may be due to the persistence of the inflammatory process or vasodilation. Perhaps the performance of a higher number of treatment sessions over time may generate a higher effect on vascularity and acne scars.

Regarding the adverse events, both procedures exhibited similar side effects. Pain demonstrated a statistically significant longer duration for microneedling, attributable to deeper tissue damage compared with laser treatment. This aspect should be communicated to patients when selecting scar treatment modalities. It is noteworthy that bleeding was reported following CO2 laser treatment, a phenomenon previously documented by Cho et al [23].

In previous studies, the occurrence of adverse effects with microneedling was rare, among erythema, pain, edema, bleeding, and hyperpigmentation are included [12,24,25]. Contraindications reported are active infections (including bacterial, viral, and fungal agents), keloidal predisposition, and immunosuppression [26]. With CO2 laser frequent adverse effects reported in the literature are pain, hyperpigmentation, and erythema. Reactivation of herpes, exudation, petechiae, pruritus, or contact dermatitis are mentioned less frequently [10]. Contraindications include active infections and patients with abnormalities of the hair follicles and sebaceous glands (which may have problems with wound healing) [27].

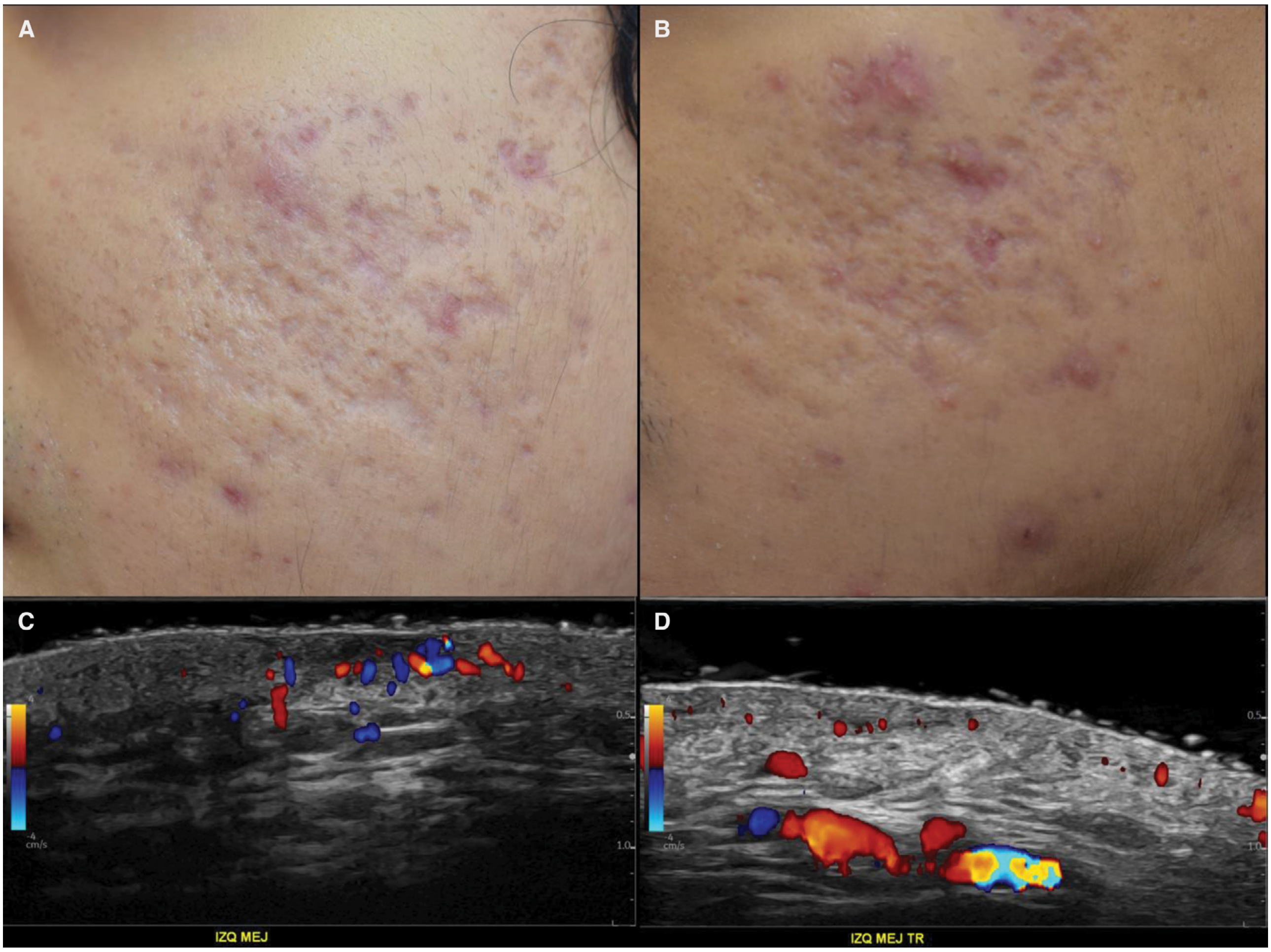

Figure 2. Effects on the left cheek with microneedling. (A) Baseline clinical image; (B) Follow-up clinical image at 3 months. Clinically, there is a decrease in the depths of acne scars. Color Doppler high-frequency ultrasound at 24 MHz: baseline (C) and follow-up (D). Notice the decrease in the degree of vascularity (colors) at follow-up.

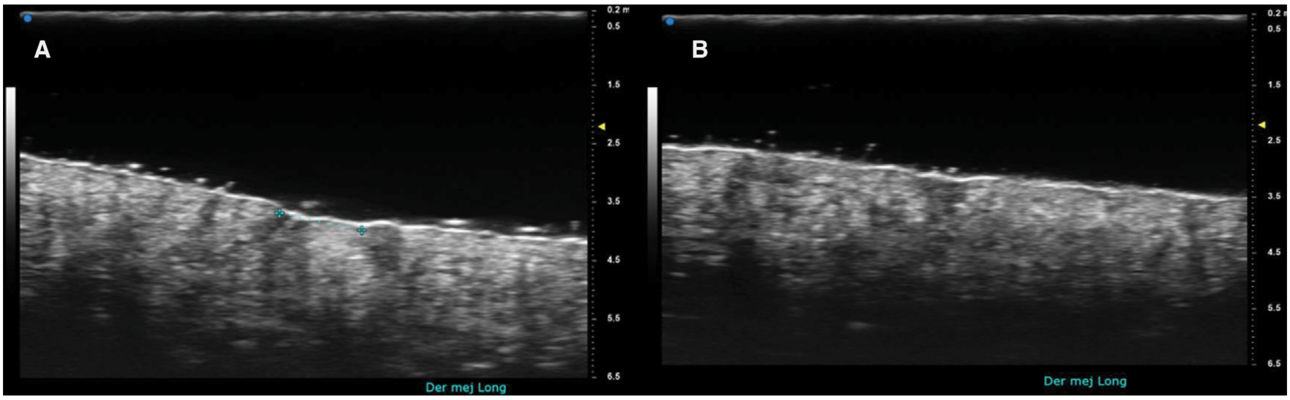

Figure 3. Ultra-high-frequency ultrasound (70 MHz; greyscale) of a scar (epidermal depression) treated with a CO_2 laser (longitudinal diameter): (A) Baseline: 1.4 mm. (B) Follow-up at 3 months: 1.3 mm.

One of the limitations of our study is the small sample size and number of evaluators, which may explain the lack of statistical significance in our results. It should be noted that the ultrasound measurement was performed on a limited number of scars and not on all of them, as we did with the ECCA scale and patient satisfaction survey. Also, the evaluators may have variations in the count of scars between both sessions, which can also affect the final results, as shown in previous studies [28]. One point to highlight is the number of sessions, as several clinical studies on microneedling suggest higher effectiveness with a minimum of 3 treatment sessions [19]. This was not possible in our sample due to budget restrictions; however, it may generate a piece of evidence for the treatments using our setting. Another limitation corresponds

Table 5. Adverse Events After Both Treatments

Variable	Treatment	Mean Days	Standard Deviation	T-Test/ W Test	P-Value
Pain	Microneedling	6,78	2,44	0,002	0,0005
	CO2 laser	1,33	0,47		
Erythema	Microneedling	3,78	0,79	6,139	0,4067
	CO2 laser	8,11	8,81		
Bleeding	Microneedling	1	0,47	5,859	0,195
	CO2 laser	1,33	0,47		
Hyperpigmentation	Microneedling	34,44	39,59	7,252	0,8221
	CO2 laser	26,89	34,9		

to the exact selection of clinical and ultrasound measurement sites, since they correspond to operator-dependent methods, which, despite having performed photographic skin marking, could be susceptible to localization error given the high number of scars in these patients.

In conclusion, our study shows that high and ultra-high-frequency color Doppler ultrasound can detect, measure, and assess regional vascularization at the scar regions, without significant differences between microneedling and CO_2 laser treatment. Hence, it should be considered a possible tool for monitoring treatment response. Clinical improvement can be detected with both methods, which suggest them as viable options for treating atrophic acne scars. Further studies on a larger number of patients and longer follow-up duration are needed to obtain more accurate data.

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