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Efficacy and safety of sequential treatment with botulinum toxin type A, fractional CO2 laser, and topical growth factor for hypertrophic scar management: a retrospective analysis

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Hypertrophic scars arise from aberrant wound healing and can lead to functional and aesthetic impairments. One of the common interventions for treating hypertrophic scars is fractional carbon dioxide (CO2) laser, which employs narrow laser beams to stimulate dermal collagen deposition. Recent studies and reports have suggested that combining laser therapy with other interventions such as botulinum toxin type A (BTX-A) and topical growth factors may enhance treatment outcomes. Here, we examine the efficacy and safety of a sequential combination of BTX-A, fractional CO2 laser, and topical growth factors, referred to as combined therapy, for treating hypertrophic scars compared with only using fractional CO2 laser and topical growth factors, referred to as monotherapy. Our retrospective study includes 128 patients with hypertrophic scars (56 underwent monotherapy and 72 underwent combined therapy), which were followed-up for up to 15 months after the initiation of treatment to collect demographic and clinical data. Our analysis showed that the combined therapy significantly outperformed monotherapy in improving Vancouver scar scale scores ($P < 0.05$) and in the reduction of scar thickness ($P < 0.05$), without increasing adverse complications. Repeated treatments further augmented the efficacy of the combined therapy. Subgroup analysis revealed that combined therapy was notably more effective in reducing Vancouver scar scale scores and scar thickness in early-stage scars compared to late-stage ($P = 0.023$ and $P = 0.045$, respectively). Our study suggests that including BTX-A treatment before fractional CO2 laser and topical growth factors offers superior efficacy in reducing hypertrophic scars. We encourage early intervention and repeated treatments for optimal treatment outcomes.

Keywords Fractional CO2 laser, Deep and active FX modes, Botulinum toxin type A, Hypertrophic scar, Retrospective study, Monotherapy, Combined therapy

A hallmark of wound healing involves the secretion of collagen, which is essential for closing injuries[1–4]. However, irregulated collagen deposition can lead to the formation of excessive scars. Hypertrophic scar is a type of excessive scar often resulting from surgeries, burns, and trauma[5]. Its incidence varies by injury type[6] and is affected by factors such as age, wound infection, gender, genetic background, site, and injury depth[7]. While hypertrophic scars are typically confined to the original site and may fade over time[8,9], their undesirable

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appearance, itchiness, and possible recurrence still cause severe aesthetic concerns and psychological distress[10,11]. Conventional treatments for hypertrophic scars, including Pulsed Dye Laser, Nd: YAG Laser, compression therapy, silicone gels, topical and intra-lesional 5-fluorouracil, and steroid injections, are sometimes effective. However, their use is often limited to minimize side effects[12–17]. Hence, safe and effective medical interventions are in demand for the patient's mental and physical health.

One promising approach for scar removal and skin rejuvenation is fractional CO2 laser therapy, which applies a laser at a wavelength of 10,600 nm to specifically target the epidermis or dermal papillary layer[18,19]. Recent studies have proven the effectiveness of the fractional CO2 laser, especially when applied in a combination of Deep and Active FX modes[20,21]. Despite these advancements, repeated use of this therapy may lead to complications such as post-inflammatory hyperpigmentation, prolonged erythema, skin swelling, and infection[22,23]. Thus, enhancing the efficacy of fractional CO2 laser without increasing adverse reactions, has become a critical research area in dermatology.

Including topical growth factors in fractional CO2 laser treatment can increase efficacy. These factors are a class of secreted polypeptide ligands widely used for the treatment of burns, chronic wounds, fresh wounds, and repair of corneal lesions[24,25]. One successful example is the usage of recombinant bovine basic fibroblast growth factor (rbFGF) and fractional CO2 laser in Acne Scars[26].

Besides topical growth factors, antifibrotic agents have also been used to enhance the treatment of hypertrophic scars, particularly those that are thicker[27,28]. These agents includes Fluorouracil (5-FU)[29,30], Verapamil Hydrochloride[30], and Bleomycin[31]. Besides, this approach may also reduce side effects associated with fractional CO2 laser. For instance, triamcinolone, commonly used for treating skin itching and inflammation, has been successfully used with fractional CO2 laser for safer and more effective scar treatments[32,33].

BTX-A is another agent showing promise when combined with a fractional CO2 laser[34–37]. It alleviates scar tension by causing muscle fiber atrophy[38] and has shown effectiveness in treating muscle spasms, facial wrinkles, pathological scarring, and analgesia[21,39]. Recent studies have indicated that both topical and injected forms of BTX-A can be combined with laser therapy to offer improved therapeutic outcomes [34–37, 40–42]. However, there is uncertainty regarding the order and timing of BTX-A and laser therapy when given in combination, as evidence suggests there are no significant outcome differences whether BTX-A is administered before or after laser treatments[40,41,43,44]. A recent comprehensive review recommends using BTX-A before laser treatment to minimize the discomfort of injecting BTX-A into more sensitive skin following laser treatment[45]. Nevertheless, the effectiveness and safety of this sequential combined therapy in treating hypertrophic scars remain unexplored.

In this retrospective study, we evaluate the safety and efficacy of a sequential combination of BTX-A, fractional CO2 laser, and rbFGF, referred to as combined therapy, for treating hypertrophic scars in 128 patients compared with only using fractional CO2 laser and rbFGF, referred to as monotherapy. Our goal is to show the effect of the pre-treatment with BTX-A for hypertrophic scar treated with fractional CO2 laser and rbFGF.

Methods

Patient selection

This retrospective study was conducted at the General Hospital of Ningxia Medical University, Yinchuan, China, from January 2022 to February 2024. A total of 128 patients with hypertrophic scars from various causes were included: 56 received fractional CO2 laser monotherapy, while 72 underwent combined therapy with fractional CO2 laser and BTX-A. In the combined therapy group, intradermal injection of BTX-A was administered two weeks before each fractional CO2 laser session. Patients with various types of scars, including those caused by burns, surgical procedures, chemical agents, electricity, and other factors, were included without bias. Detailed patient information is provided in Table 1. No statistical differences were observed between the groups in terms of scar location, duration since injury, or history of previous injuries. Patients underwent one to three treatment sessions based on their individual improvement. Inclusion criteria were individuals aged 18 to 70 years without serious underlying conditions, while exclusion criteria included patients under 18 or over 70, those with a history of photosensitivity, skin tumors, or abnormal mental states.

All participants provided written informed consent (supplementary material). Demographic and clinical data such as age, gender, scar etiology, scar location, time since scar formation, number of treatments, and intervals between treatments were collected with the approval of the Ethics Committee of the General Hospital of Ningxia Medical University (No.: KYLL-2021-601). All methods were performed in accordance with the relevant guidelines and regulations.

Fractional CO2 laser treatment with deep and active FX modes

Representative images of hypertrophic scars before and immediately after fractional CO2 laser treatments are provided in Supplementary Fig. 1. Prior to treatment, the scar area was cleansed and anesthetized with Lin compound lidocaine cream (Guoyao Zhunzi, H36022084, Beijing Unisplendour Pharmaceutical Co., Ltd.), formulated at 10 g:50 mg. After a 30-minute application, the cream was removed, and the area was disinfected. Fractional CO2 laser treatment was performed using the AcuPulse Fractional King device (Lumenis Medical Laser Company, USA). A preliminary scan was conducted using Deep FX mode, with settings of 15 to 20 mJ energy, a 10 mm spot diameter, and a spot density of 3–5%. Scars were categorized by thickness as mild, medium, or heavy, and treatment parameters were adjusted accordingly. Medium and heavy scars were treated with a 2 mm spot diameter, 100 mJ energy, and 40% spot density, while mild scars were treated with a 10 mm spot diameter, 15 to 20 mJ energy, and 10–15% spot density. This classification only determined laser parameters, following hospital standard procedures. Treatment duration ranged from 10 to 30 min.

After treatment, ice was applied to the treated area for 30 to 40 min, followed by recombinant bovine basic fibroblast growth factor (rbFGF) gel (Zhuhai Yisheng Biopharmaceutical Co., Ltd., approval number: S20020113)

Demographics	monotherapy	Combined therapy	*P* value
Number	56	72	
Age, year	32.3 ± 15.9	35.4 ± 16.7	0.082
Gender, n (%)			0.581
Male	38 (67.8)	49 (68.1)	
Female	18 (32.2)	23 (31.9)	
Interval between treatments, month	3.0 ± 0.8	2.7 ± 1.1	0.225
Time of scar formation, month	6.3 ± 3.5	7.1 ± 4.2	0.619
Etiology of scars, n (%)			0.785
Burn	30 (53.6)	45 (62.5)	
Surgical procedures	8 (14.3)	10 (13.9)	
Chemical agent	6 (10.7)	7 (9.7)	
Electricity	5 (8.9)	6 (8.3)	
Other	7 (12.5)	4 (5.6)	
Location of scars, n (%)			0.955
Head and/or neck	25 (44.6)	32 (44.4)	
Trunk	12 (21.4)	17 (23.6)	
Extremity	19 (33.9)	23 (31.9)	
Number of treatments, n (%)			0.637
1	29 (51.8)	31 (43.1)	
2	12 (21.4)	25 (34.7)	
> 2	15 (26.8)	16 (22.2)	

Table 1. Overview of participant demographic data according to therapy.

applied twice daily for a week to promote wound healing. Each application included 25,000 IU (50 µg) per 5 g. Patients were advised to keep the treated area clean and dry for the first three days, avoid facial washing, skincare products, makeup, and protect the skin from sun exposure. Patients underwent different sessions, from one to three, according to the improvement. The average interval between sessions is three months. The decision to schedule a subsequent treatment is contingent upon the evaluation of the results from the previous session.

BTX-A treatment

Neuronox, supplied as a 100 U vacuum-dried powder in a single-use vial, was prepared by reconstituting with 2 ml of sterile, preservative-free 0.9% saline, achieving a concentration of 5 U/0.1 ml (#S10970037; 100 U; produced by Hengli, in Lanzhou, China). Lesions were treated with an intradermal injection of 5 IU/cm² at 2 weeks before each session of fractional laser treatment. The decision to inject BTX-A before laser treatment was made according to the recommendation of the recently published study to minimize the discomfort of injecting BTX-A into the more sensitive laser-treated skin[45]. A 2-week interval between treatments was selected to avoid potential inactivation of BTX-A by laser. The injection was administered intradermally at the periphery before targeting the body of the scar. Follow-ups were scheduled at one- and six months post-injection. The decision to use BTX-A was made by the dermatologist based on a comprehensive evaluation of the scar, the patient's willingness, and any known allergies to the treatment.

Assessments of effectiveness and safety

Patients were followed up on an outpatient basis for up to 15 months with an average of 7.2 ± 2.2 months. Scar treatment was evaluated at 1 month after each treatment session. Scar treatment efficacy was gauged using the Vancouver scar scale, which includes pigmentation (0–3), vascularity (0–3), pliability (0–5), and height (0–4), for a maximum score of 15, and changes in scar thickness, measured by high-frequency ultrasound. To evaluate treatment safety, adverse events such as itching, pain, discharge, bleeding, and swelling were recorded after the completion of all treatment sessions.

Ultrasound examination

Prior to the examination, the subject's scar area was cleansed, followed by the initiation of a high-frequency ultrasound examination (ULTIMUS[7P], VINNO). Initially, a trained medical professional applied a specialized ultrasound gel to the center of the scar. Subsequently, the skin ultrasound probe, operating at a frequency of 20 MHz and positioned perpendicular to the subject's skin surface, was gently maneuvered over the scar area. Particular care was taken to ensure minimal pressure was applied to the subject's skin during the procedure. For each part, three ultrasound images were captured. vascularity, the thickness of the scar, and pliability in each image were measured. The average value of these measurements was calculated. Next, the ultrasound images of both normal skin and scar tissue were processed using the DFY-1 ultrasound image diagnosis and analysis software to analyze the characteristics of the scar. Three distinct areas were selected within each image to measure the echo intensity, and the average value of these measurements was computed.

Subgroup analysis

Subgroup analyses were conducted to examine variations in the efficacy and safety of combined therapy among scar patients at distinct stages. Patients were divided into early-stage (treatment within six months post-injury) and late-stage (treatment after six months post-injury) groups. The effectiveness and safety of the combined therapy in these distinct subgroups were then compared.

Statistical analysis

The statistical analysis in this study was conducted utilizing SPSS version 20.0. similar to previous studies[46,47]. Continuous variables were presented as mean with standard deviation (SD) $(\overline{\chi} \pm s)$. Comparisons between groups and subgroups were tested for significance using the two-tailed Mann-Whitney U test. Categorical data were presented as frequencies and percentages and assessed with chi-square χ^2 tests. P value < 0.05 was considered indicative of statistical significance.

Results

Demographic overview of therapy groups

A total of 249 patients admitted for hypertrophic scar treatment were initially selected for this study (Fig. 1). Of those, 121 were excluded based on the predetermined criteria. Among the 128 patients included in the study, 56 received monotherapy while 72 received the combined therapy. Eighty-seven of the participants were male. The predominant cause of hypertrophic scars was burns. The most common locations for scars were the head and/or neck, followed by the extremities and trunk.

A demographic overview of the mono- and combined therapy groups is summarized in Table 1. The average patient' age was 32.3 ± 15.9 for the monotherapy group and 35.4 ± 16.7 for the combined therapy group. The average duration since scar formation was 7.1 ± 4.2 months for the monotherapy group and 6.3 ± 3.5 months for the combined therapy group. Treatment intervals were 3.0 ± 0.8 months and 2.7 ± 1.1 months for the mono-

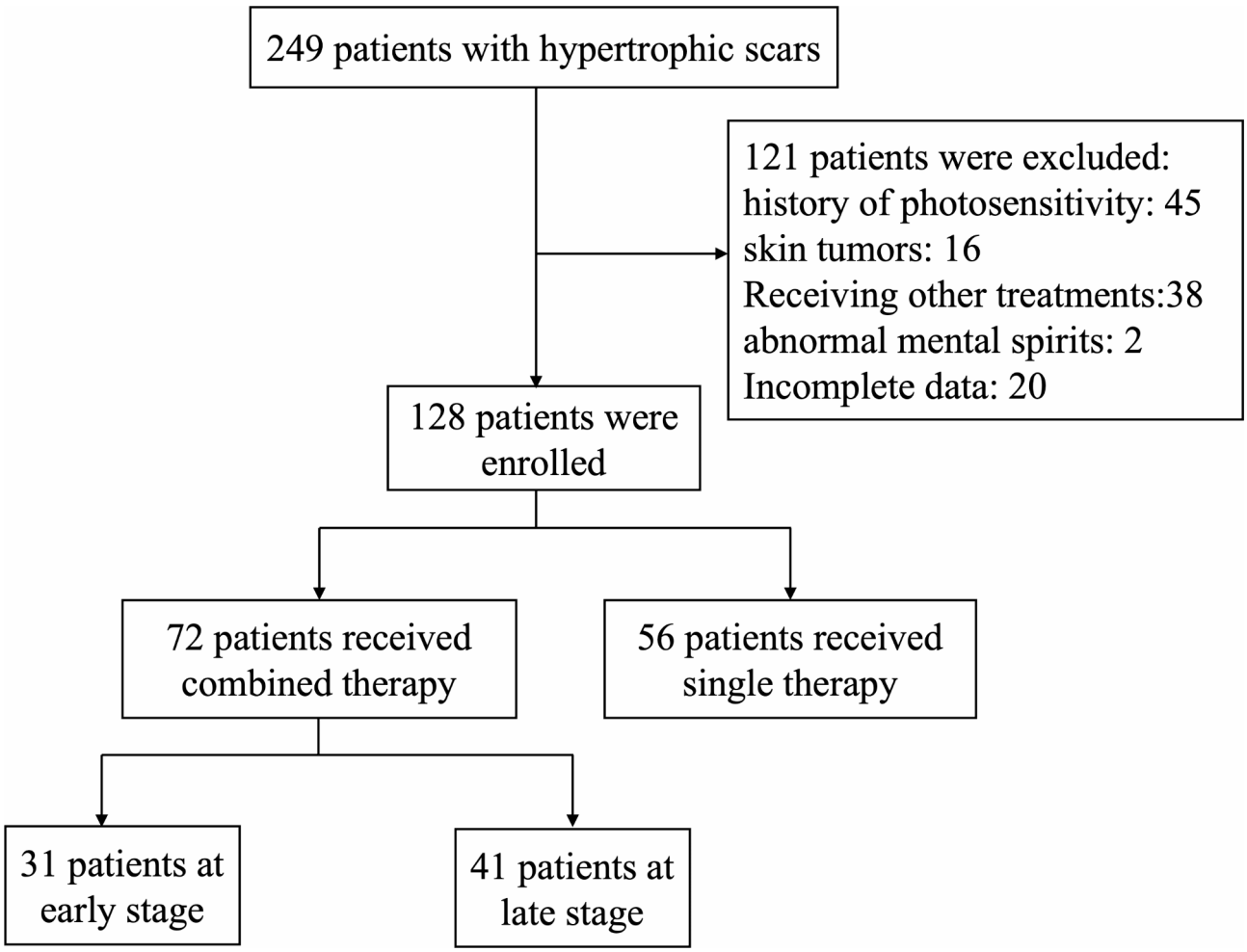

Fig. 1. Trial profile. A sketch showing the patients involved in this study. Inclusion criteria: individuals aged between 18 and 70 years with no serious underlying conditions. Exclusion criteria: individuals under 18 or over 70 years of age, those with a history of photosensitivity, individuals with skin tumors, and those presenting with abnormal mental states.

and combined therapy groups, respectively. In the monotherapy group, 29 participants underwent 1 treatment, 12 had 2 treatments, and 15 had 3 treatments. In the combined therapy group, 31 participants underwent 1 treatment, 25 had 2 treatments, and 16 had 3 treatments. Overall, no significant demographic differences were observed between the two groups (Table 1).

Combined therapy is more effective in shrinking hypertrophic scars

We first assess the efficacy of mono- versus combined therapy in shrinking hypertrophic scars. We employed the Vancouver scar scale score and measured changes in scar thickness. Both therapies demonstrated a significant reduction of hypertrophic scars, with P values of 0.042 for monotherapy and 0.001 for combined therapy (Table 2; Figs. 2 and 3). Before treatments, the two groups have no significant differences in scar scale and thickness ($P > 0.05$). However, after treatment, patients receiving combined therapy have notably smaller scars with reduced scale, pigmentation, vascularity, pliability, height, and thickness ($P < 0.05$) (Table 2). Accordingly, our result suggested combined therapy to be more effective in reducing hypertrophic scars (Fig. 4).

Combined therapy is as safe as monotherapy

We then evaluate the safety of mono- and combined therapy by analyzing the incidence of adverse complications among patients (Table 3). Overall, there was no significant difference in the occurrence of adverse complications between patients receiving monotherapy (62.5%, 35 cases) and those undergoing combined therapy (68.1%, 49 cases). For both groups, the most common complication was pruritus (monotherapy, 21.5%; combined therapy, 23.6%), followed by seepage (monotherapy, 17.8%; combined therapy, 15.3%), bleeding (monotherapy, 12.5%; combined therapy, 13.9%), swelling (monotherapy, 5.3%; combined therapy, 8.3%), and pain (monotherapy, 5.3%; combined therapy, 6.9%). Taken together, these results indicate that combined therapy is as safe as monotherapy while demonstrating enhanced effectiveness in reducing hypertrophic scars.

Combined therapy is enhanced by repeated treatment

To further evaluate the effectiveness of combined therapy, we assessed the performance of combined therapy over multiple treatment sessions. Our results indicate that patients receiving combined therapy typically showed a significant reduction in the Vancouver scar scale after two treatments, with improvements gradually increasing thereafter (Fig. 5A). On the other hand, the majority of the reduction in scar thickness occurred after the initial treatment (Fig. 5B).

Demographics	Monotherapy	Combined therapy	*P* value*
Total score of Vancouver scar scale			
Before	9.4 ± 3.5	9.8 ± 3.8	0.843
After	7.9 ± 2.1	7.2 ± 1.5	0.046
P value#	0.042	0.001	
Scar thickness, mm			
Before	7.2 ± 1.8	1	0.916
After	3.0 ± 1.1	1.9 ± 1.3	0.017
P value#	0.01	0.01	
Pigmentation			
Before	2.3 ± 0.9	2.5 ± 1.1	0.475
After	1.5 ± 0.7	1.0 ± 0.5	0.043
P value#	0.046	0.012	
Vascularity			
Before	1.9 ± 0.8	2.0 ± 0.7	0.775
After	1.4 ± 0.6	0.6 ± 0.3	0.024
P value#	0.212	0.543	
Pliability			
Before	2.2 ± 0.7	2.3 ± 0.5	0.501
After	1.7 ± 0.8	1.1 ± 0.4	0.037
P value#	0.053	0.029	
Height			
Before	2.9 ± 0.5	2.8 ± 0.7	0.713
After	2.4 ± 0.4	1.8 ± 0.5	0.015
P value#	0.087	0.045	

Table 2. Effectiveness of mono- and combined therapy for hypertrophic scars. Note: #, comparison of the Vancouver Scar Scale and scar thickness before and after the treatment; *, comparison of the Vancouver Scar Scale and scar thickness between monotherapy and combined therapy.

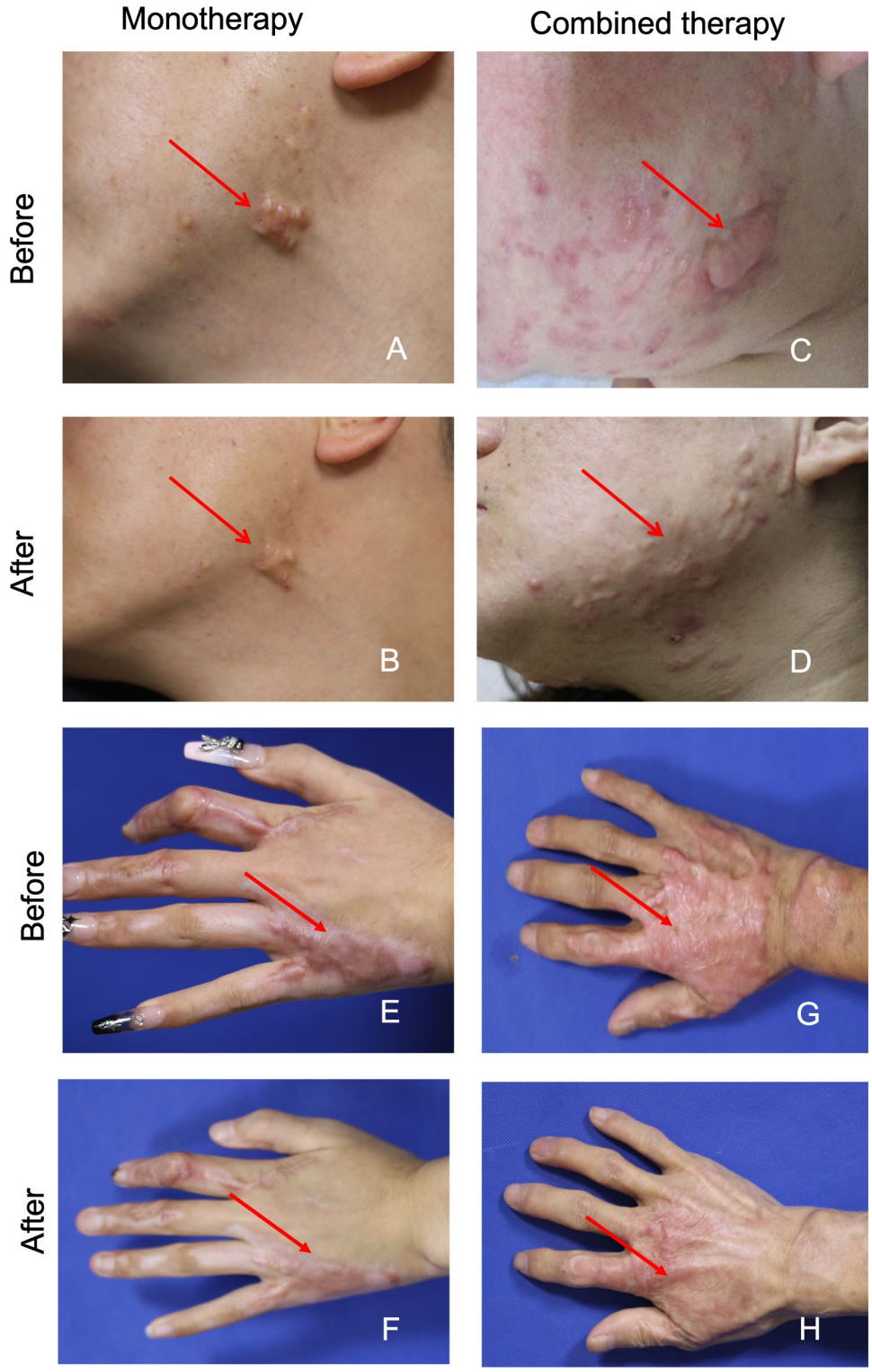

Fig. 2. Representative pictures of hypertrophic scars before and after treatments. shown as representatives. (**A**) A 25 years-old male patient with a hypertrophic scar on his neck prior to monotherapy. (**B**) The picture of the same patient in (A) after 3 sessions of monotherapy. (**C**) A 30 years-old female patient with a hypertrophic scar on her neck prior to combined therapy. (**D**) The picture of the same patient in (**C**) after 3 sessions of combined therapy. (**E**) A 43 years-old female patient with a hypertrophic scar on her hand prior to monotherapy. (**F**) The picture of the same patient in (**E**) after 3 sessions of monotherapy. (**G**) A 55 years-old female patient with a hypertrophic scar on her hand prior to combined therapy. (**H**) The picture of the same patient in (**G**) after 3 sessions of combined therapy. Picture was taken at 1 month after the last treatment. Red arrows indicate the hypertrophic scars. Pictures in (**A**, **C**, **E**, **G**) were taken prior to the initial treatment. Pictures in (**B**, **D**, **F**, **H**) were taken at 1 month after the last treatment session. The use of pictures has been approved by the patients.

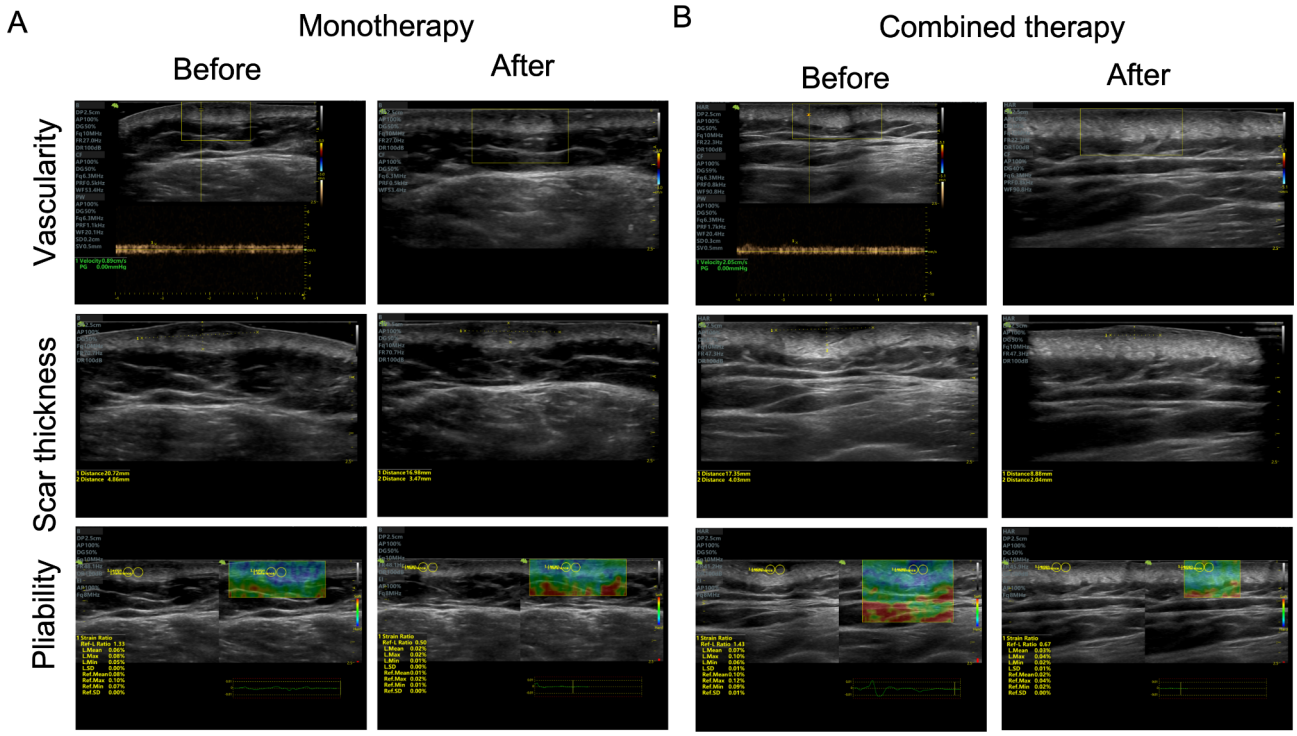

Fig. 3. Representative pictures of ultrasound measurements before and after treatments. (**A**) Representative images showing the ultrasound measurements of vascularity, scar thickness, and pliability of the hypertrophic scar on the neck from a 25 years-old male patient before and after 3 sessions of monotherapy. (**B**) Representative images showing the ultrasound measurements of vascularity, scar thickness, and pliability of the hypertrophic scar on the neck from a 30 years-old female patient before and after 3 sessions of combined therapy. Pictures were taken either prior to the initial treatment (before) or at 1 month after the last treatment session (After). The use of pictures has been approved by the patients.

Combined therapy performs better at the early stages after scar formation

We next evaluated the performance of combined therapy in early- and late-stage scars. We stratified patients receiving combined therapy into two subgroups: an early-stage subgroup and a late-stage subgroup based on the time after scar formation. Notably, there are no significant demographic differences between the two groups (Table 4).

To assess the performance of combined therapy in the two subgroups, we measured the changes in Vancouver scar scale and scar thickness. As expected, combined therapy significantly shrunk hypertrophic scars in both subgroups (Table 5). While the two subgroups have no significant differences in scar scale and thickness before treatment, early-stage patients exhibit much smaller scars, as demonstrated by lower scar scale and thickness, after treatment (Table 5). Similarly, early-stage patients demonstrated a larger reduction in scar scale and thickness (Fig. 6). Together, these results suggested that combined therapy is more effective at early-stage scars, and its performance is enhanced by repeat treatments.

Discussion

In this study, we demonstrated that a sequential combination of BTX-A, fractional CO2 laser, and topical growth factors offers superior efficacy in reducing hypertrophic scars compared to only using fractional CO2 laser and topical growth factors. Our results suggest that combined therapy is particularly effective in the early stages (within 6 months) of scar formation, with its performance further enhanced by repeated treatments.

Fractional CO2 laser technology has been utilized in dermatology as a non-surgical approach for many years since 2007[48–53]. Recently, there has been a trend towards integrating fractional CO2 laser treatment with other modalities to enhance its efficacy[27]. Huang et al. combined ablative fractional CO2 laser with 5-fluorouracil ethosomal gel[29]. While effective in a rabbit model, this combined therapy did not surpass monotherapy in human patients[29]. Conversely, our study indicated that sequential combining BTX-A, fractional CO2 laser, and rbFGF significantly surpasses monotherapy in human patients (Figs. 2, 3 and 4; Tables 2 and 3). This improvement may be due to BTX-A's ability to alleviate scar tension by causing muscle fiber atrophy[38,54–57].

Unexpectedly, patients receiving the combined therapy exhibited the same rate of pruritus complications as those receiving monotherapy, even though BTX-A has been reported to alleviate itching[58]. However, given the combined therapy did not increase any complications at least, we still recommend the use of the combined therapy over monotherapy for treating hypertrophic scars, where feasible.

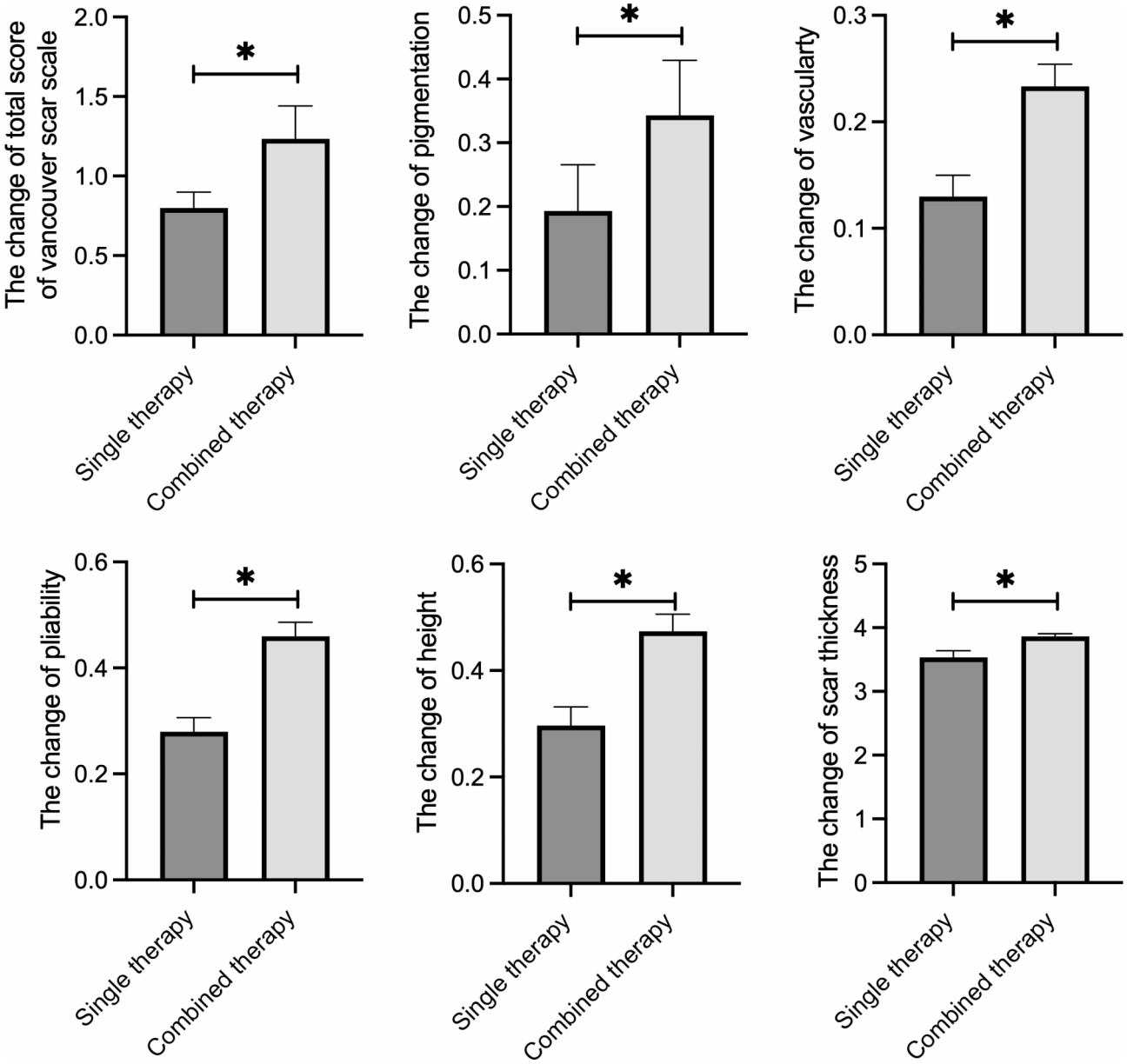

Fig. 4. Bar plots showing the reduction in hypertrophic scar size and thickness after mono- and combined therapy. Data was presented as mean with standard deviation (SD). Comparisons were made between mono- and combined therapy groups. Statistical significance was tested as mentioned in the method section. $^{*}P < 0.05$.

Demographics	Monotherapy, *n* (%)	Combined therapy, *n* (%)	*P* value
Pruritus	12 (21.2)	17 (23.6)	0.613
Pain	3 (5.3)	5 (6.9)	0.592
Bleeding	7 (12.5)	10 (13.9)	0.637
Swelling	3 (5.3)	6 (8.3)	0.595
Seepage	10 (17.8)	11 (15.3)	0.518
Total	35 (62.5)	49 (68.1)	0.722

Table 3. Adverse complications in participants receiving mono- and combined therapy.

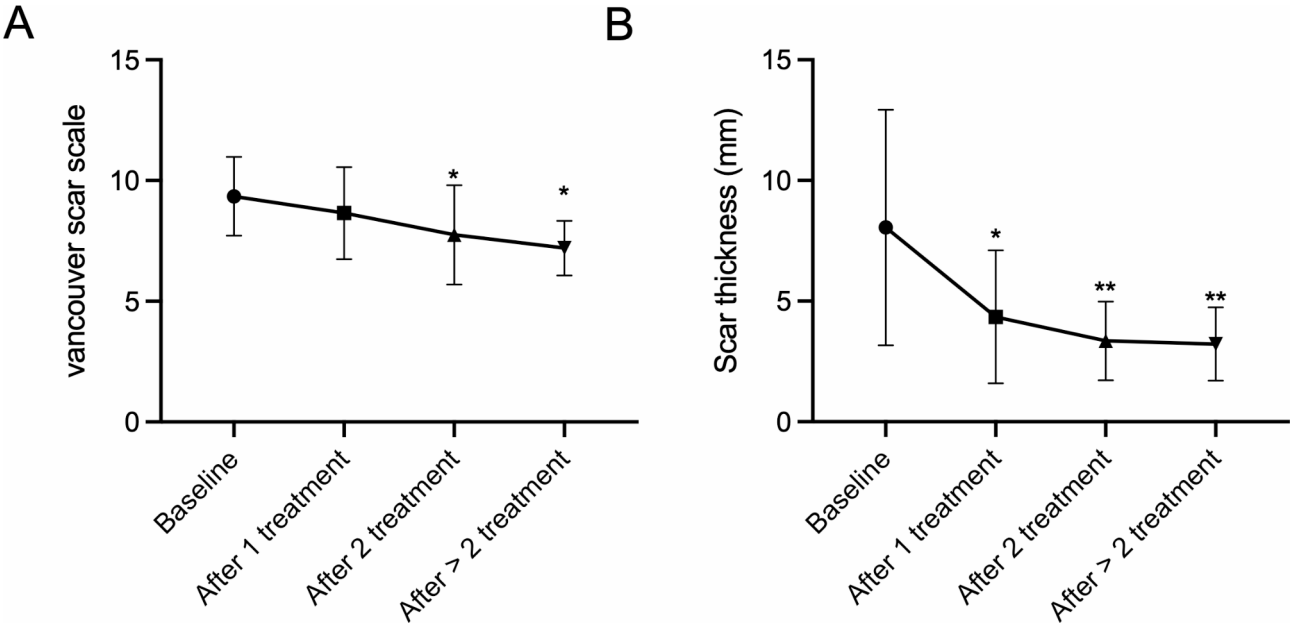

Fig. 5. Performance of combined therapy in multiple treatment sessions. (**A**) Changes in Vancouver scar scale after repeated treatments; (**B**) Changes in scar thickness after multiple treatments. Data was presented as mean with standard deviation (SD). Comparisons were by comparing each treatment to the baseline. Statistical significance was tested as mentioned in the method section. $^{*}P < 0.05$; $^{**}P < 0.01$.

Demographics	Early stage	Late stage	*P* value
Number	31	41	
Age, year	33.6 ± 12.8	34.3 ± 12.9	0.318
Gender, n (%)			0.522
Male	23 (74.2)	29 (70.8)	
Female	8 (25.8)	12 (29.2)	
Interval between treatments, month	2.4 ± 0.9	2.5 ± 1.0	0.116
Time of scar formation, month	3.7 ± 2.1	8.6 ± 2.5	0.001
Etiology of scars, n (%)			0.276
Burn	22 (70.1)	27 (65.9)	
Surgical procedures	4 (12.9)	8 (19.5)	
Chemical agent	3 (9.6)	4 (9.7)	
Electricity	1 (3.2)	2 (4.9)	
Other	1 (3.2)	0 (0.0)	
Location of scars, n (%)			0.325
Head and/or neck	16 (58.9)	15 (36.6)	
Trunk	5(17.6)	10 (24.4)	
Extremity	10 (23.5)	16 (39.0)	
Number of treatments, n (%)			0.496
1	16 (51.6)	15 (36.5)	
2	9 (29.0)	16 (39.0)	
> 2	6 (19.4)	10 (24.4)	

Table 4. Demographic overview of patients receiving combined therapy at early and late stages.

Fibroblast growth factors play dynamic roles in fibrosis. In contrast to the long-held belief that growth factors stimulate fibroblast proliferation and activation to produce collagen, recent studies have suggested that many growth factors, such as FGF-2[59], FGF-9[60], and FGF-18[60], inhibit fibroblast activation and collagen deposition in various organs[61]. Since fibrosis is primarily driven by the expression of pro-fibrotic genes[28,62], one explanation for this dynamic is that different growth factors influence the expression of pro-fibrotic genes in distinct ways[63]. For example, FGF-2 has been shown to inhibit certain pro-fibrotic genes in both human and animal models[63]. Although the mechanism behind the antifibrotic effects of growth factors is not fully understood, many have

Demographics	Early stage	Late stage	*P* value
Total score of Vancouver scar scale			
Before	8.9 ± 1.5	9.2 ± 1.8	0.565
After	6.9 ± 1.4	7.3 ± 1.8	0.042
P value	0.001	0.002	
Scar thickness, mm			
Before	7.1 ± 2.8	6.9 ± 2.2	0.883
After	2.3 ± 0.5	3.0 ± 0.8	0.050
P value	0.001	0.001	

Table 5. Performance of combined therapy at early and late stages.

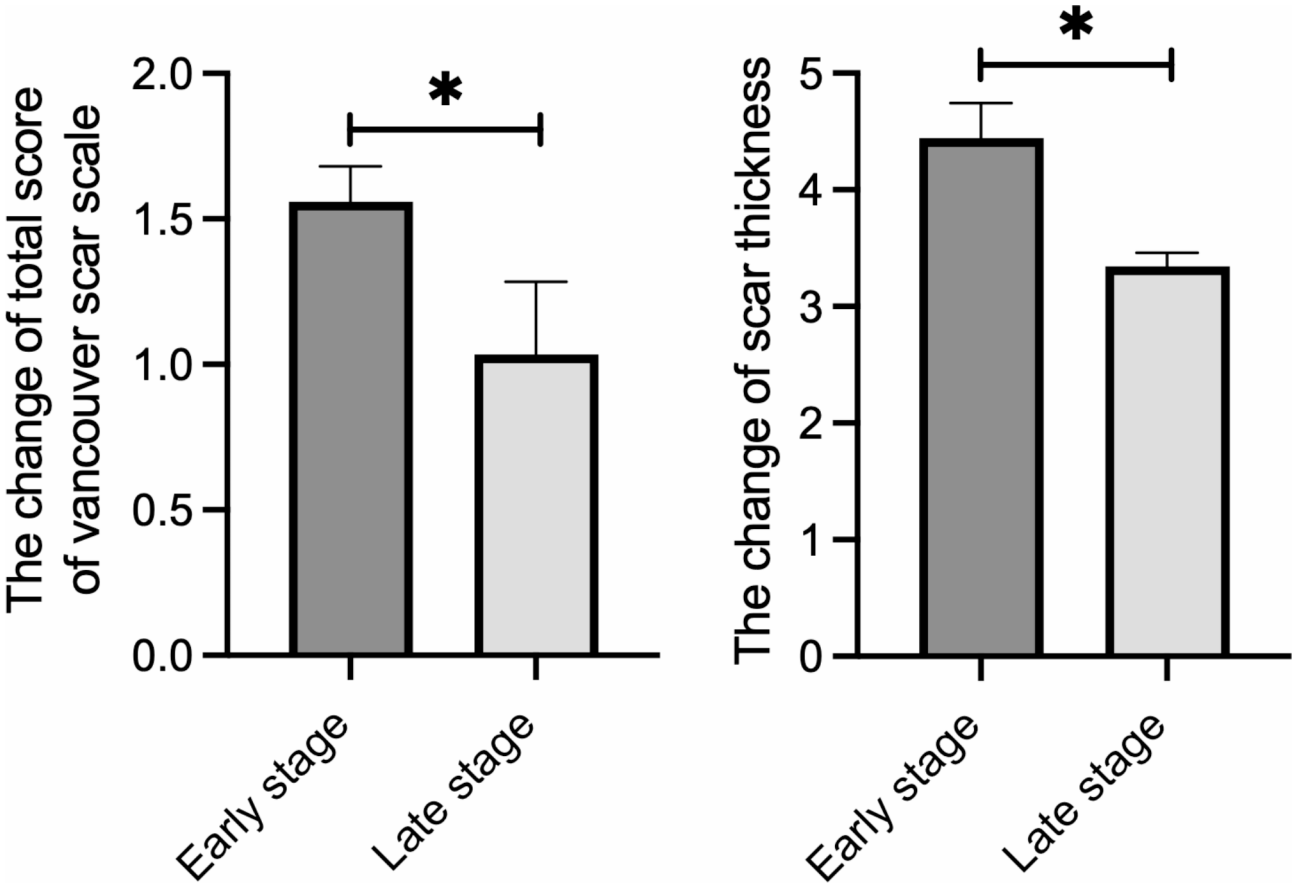

Fig. 6. Bar plots showing the reduction in hypertrophic scar size and thickness after combined therapy at early and late stages. Data was presented as mean with standard deviation (SD). Comparisons were made between early- and late-staged scars. Statistical significance was tested as mentioned in the method section. $^{*}P < 0.05$.

proven effective in treating scars[26]. In this regard, we recommend the inclusion of growth factors, such as rbFGF, in the treatment of hypertrophic scars.

The performance of the combined therapy improves with repeated treatments. We observed a gradual reduction in the scar scale after at least two repeated treatments (Fig. 5A), which aligns with the published notion by Kemp Bohan et al.,[64]. Interestingly, scar thickness decreased dramatically after the initial treatment (Fig. 5B), which contrasts with findings that two laser treatments are necessary to reduce scar thickness[64]. This difference may be due to the incorporation of BTX-A and may warrant future investigation. Furthermore, we noted that combined therapy is more effective in patients within six months of scar formation. This finding is consistent with published research[65]. Based on these, we recommend clinicians apply at least two repeated treatments of combined therapy at early stages to achieve optimal outcomes.

Although our study shows promise, it is important to recognize certain limitations. First, the hypertrophic scars analyzed were caused by various etiologies, which might have influenced the effectiveness of therapies. Consequently, focused studies on hypertrophic scars with uniform etiology may be a better choice. However,

such studies could be very challenging as limiting the study to a single etiology might significantly reduce the available sample size. Second, the majority of participants involved in the study received treatment within 12 months post-injury. Future studies may be needed to explore the performance of combined therapies on older scars.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

All authors (JW, LH, LL, and JL) contributed to the conception and study design. JW, LH, JL, RX, YY, TG, TH, YW, and JZ coordinated and managed all experiments of the study. CD carried out the literature search. JW, LH, JL, RX, YY, and JZ conducted data collection and performed preliminary data preparations. JW, LH, and JL conducted data analyses and all the authors contributed to the interpretation of data. FJ, HL, LL, and LW wrote the draft of the paper and all authors provided substantive feedback on the paper and contributed to the final

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