Universitat Autònoma de Barcelona

“Combined Fractional LASERs Resurfacing with Platelets Rich Plasma for treating Post-acne atrophic scarring”

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"Combined Fractional LASERs Resurfacing with Platelets Rich Plasma for treating Post-acne atrophic scarring"

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The present work has been carried out in collaboration between the Dermatology Services of the Santa Creu and Sant Pau Hospital in Barcelona, the DEKA M.E.L.A. S.r.l. in Florence, and the Cosmetic Surgery Clinic in Kuwait.

Each of the researchers who participated in this study has read the protocol and agreed to coordinate this clinical trial in accordance with all the stipulations established in it, complying with the Helsinki Declaration, and the Manual of Standards for Good Clinical Practice and the relevant national regulations.

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Dedicated to my Father, Abdelsalam, my Mother, Huda,
my wife, Nada, and my little princess Perla.

Abbreviations:

AC	Absorption Coefficient
ACD-A	Acid Citrate Dextrose
AFL	Ablative Fractional Laser Resurfacing
AFT	Autologous Fat Transfer
ASCs	Adipose Stem Cells
bFGF	basic Fibroblast Growth Factor
BMSCs	Bone Marrow Stem Cells
COL	Collagen
CO_2	Carbon Dioxide
CROSS	Chemical Reconstruction Of Skin Surface
CW	Continous Wave
DWR	Depth Width Ration
ECCA	Échelle d'évaluation Clinique des Cicatrices d'Acné
ECM	ExtraCellular Matrix
EGF	Epidermal Growth Factor
Er: YAG	Erbium:Yttrium-Aluminum-Garnet
FASQoL	Facial Acne Scar quality of Life
FCL	Fractional CO_2 Laser
Fg	Fibrinogen
FGFRs	Fibroblast Growth Factor Receptors
Fn	Fibronectin
FP	Fractional Photothermolysis
GAIS	Global Aesthetic Improvement Scale
G-CSF	Granulocyte-Colony Stimulating Factor
GFs	Growth Factors
HA	Hyaluronic Acid
IGF-1	Insulin-like Growth Gactor-1
IL-8	Interlukin-8
KLCs	Keratinocyte Like Cells
LIOB	Laser-Induced Optical Breakdown
L-PRF	Leucocyte – Platelet Rich Fibrin
L-PRP	Leucocyte – Platelet Rich Plasma
MLA	Micro Lens Array
MMPs	Matrix MetalloProteinases
MSCs	Mesenchymal Stem Cells
MTZ	MicroThermal Zone
NAFL	Non- Ablative Fractional Laser Resurfacing
Nd: YAG	Neodmium:Yttrium-Aluminum-Garnet
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NSS	Normal Saline Solution
PAI-1	Plasminogen Activator Inhibitor type I
PDGF	Platelet-Derived Growth Factor
PDL	Pulsed-Dye Laser
PIH	Post-Inflammatory Hyperpigmentation
PLLA	Poly-L-Lactic Acid
PPP	Platelet Poor Plasma
P-PRF	Pure-Platelet Rich Fibrin

Abbreviations

P-PRP	Pure-Platelet Rich Plasma
PRP	Platelet Rich Plasma
PSR	PicroSirus Red
RF	RadioFrequency
RTD	Residual Thermal Damage
SC	Stem Cells
SCARS	Self-assessment of Clinical Acne-Related Scars
SCAR-S	Scale for Acne Scar Severity
SP	Short Pulse
SR	Sublative Rejuvenation
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TCA	TriChloroacetic Acid
TEWL	Trans Epidermal Water Loss
TGF	Transforming Growth Factor
TIMPs	Tissue Inhibitors of MMPs
TLR-2	Toll Like Receptor-2
TSP-1	Thrombospondin-1
VAS	Visual Analog Scale
VEGF	Vascular Endothelial Growth Factor
Vn	Vitronectin
VSP	Variable Square Pulse
XLP	eXtraLong Pulse
YSGG	Yttrium Scandium Gallium Garnet

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SUMMARY

Summary

Purpose: To evaluate the efficacy of LASERs combination (AFL; Fractional CO2 and NAFL Fractional Er: Glass); if applied simultaneously followed by PRP for treatment of atrophic post-acne scar types. We hypothesize that this treatment combination would maximize improvement and minimize the previously reported adverse reactions for LASERs combination, especially if confining the AFL pulses and PRP injections only to the atrophic post-acne scars while applying NAFL for the entire face.

Methods: A prospective, interventional, observer-blinded, intra-patient, single center study. Patients 18 years or older with atrophic post-acne scars were recruited after applying inclusion and exclusion criteria.

All patients had 3 excision biopsies from the three most marked scars immediately after the first treatment. One biopsy of an area neither LASERs nor PRP where used served as a control. The second biopsy site was treated only by LASERs combination, while the third biopsy was treated by both LASERs combination and PRP. Patients continued to receive another 3 similar treatment sessions of combined LASERs and PRP on monthly intervals, without biopsies, and were then followed up for another 12 weeks (week 24).

Main outcomes were patients' self-assessment tools; SCARS and FASQoL, at week 4 and blind assessors' evaluation for 2 sets of digital photographs at week 24. While secondary outcomes were blinded histopathological and immunohistochemical evaluations for the biopsies of the three groups.

Results: Thirty-two patients were recruited. Only one patient was withdrawn. At week 4, FASQoL pre-treatment values were significantly lower than posttreatment values (20.64$\pm$9.76 vs 29.41$\pm$9.14, $p< 0.0001$). At week 24, only 1 patient was highly satisfied, 10 patients were satisfied, 19 patients were slightly satisfied, and 1 patient was poorly satisfied. Patients' self-assessment of Atrophic Acne Scars VAS scores at weeks 0, 4, and 24 showed that the median score among the three assessments differed significantly ($p< 0.0001$).

The mean blind assessors' pre-treatment values for atrophic acne scars score were significantly higher than posttreatment values (5.5$\pm$1.81 vs 3.15$\pm$1.41, $p< 0.0001$). The blind assessors

graded the final improvement of 2 patients as highly satisfactory, 8 patients as satisfactory, 18 patients as slightly satisfactory, and 3 patients were unsatisfactory.

By Picrosirius red stain, we reported a significant rise in collagen (COL) III ratio (new immature COL) among control, Lasers + NSS and, Lasers + PRP groups (13.9 ± 15.97 vs 24.17 ± 21.67 vs 34.41 ± 23.57, respectively, $p< 0.0001$). On the other hand, we reported a simultaneous significant decrease in COL I ratio (old mature COL) among control, Lasers + NSS and, Lasers + PRP groups (84.77 ± 15.6 vs 75.05 ± 21.05 vs 64.5 ± 23.13, respectively, $p< 0.0001$).

Conclusions: Combinations of AFL, NAFL, and PRP for treatment of post-acne atrophic scars yield earlier significant improvements when used simultaneously from the first session. This triple combination enhanced the scar remodeling with minimal adverse effects based on patients´ self-assessment 4 weeks after the first session.

Summary

Resumen

Propósito del estudio: Evaluar la eficacia de la combinación de LASER (AFL; Fractional CO2 y NAFL Fractional Er: Glass); si se aplica simultáneamente seguido de PRP para el tratamiento de los tipos de cicatrices atróficas del acné. Presumimos que esta combinación de tratamiento maximizaría la mejora y minimizaría las reacciones adversas previamente informadas para la combinación de LASER, especialmente si se limitan los pulsos de AFL y las inyecciones de PRP solo a las cicatrices atróficas del acné mientras se aplica NAFL en toda la cara.

Material y Métodos: Un estudio prospectivo, intervencionista, ciego al observador, intrapaciente, unicéntrico. Se reclutaron pacientes de 18 años o más con cicatrices atróficas post-acné después de aplicar los criterios de inclusión y exclusión.

Todos los pacientes fueron sometidos a biopsias de escisión de las tres cicatrices más marcadas inmediatamente después del primer tratamiento. Una biopsia de un área donde no se utilizaron ni LASER ni PRP sirvió como control. El segundo sitio de la biopsia se trató solo con la combinación de LASER, mientras que la tercera biopsia se trató con la combinación de LASER y PRP. Los pacientes continuaron recibiendo otras 3 sesiones de tratamiento similares de LASER y PRP combinados en intervalos mensuales, sin biopsias, y luego se les dio seguimiento durante otras 12 semanas (semana 24).

Los resultados principales fueron las herramientas de autoevaluación de los pacientes, SCARS y FASQoL, en la semana 4 y evaluación de 2 juegos de fotografías digitales en la semana 24 por 4 evaluadores a ciegas Los resultados secundarios fueron evaluaciones histopatológicas e inmunohistoquímicas a ciegas de las biopsias de los tres grupos de tratamiento.

Resultados: Se reclutaron treinta y dos pacientes. Solo se retiró un paciente. En la semana 4, los valores previos al tratamiento de FASQoL fueron significativamente más bajos que los valores posteriores al tratamiento (20,64 + 9,76 frente a 29,41 + 9,14, p <0,0001). En la semana 24, solo 1 paciente estaba muy satisfecho, 10 pacientes estaban satisfechos, 19 pacientes estaban ligeramente satisfechos y 1 paciente estaba poco satisfecho. La autoevaluación mediante EAV por parte de los pacientes de las cicatrices atróficas de acné en las semanas 0, 4 y 24 mostró que la puntuación media en las tres evaluaciones difería significativamente (p <0,0001).

Los valores medios de la puntuación de las cicatrices atróficas de acné pretratamiento por los evaluadores a ciegas fueron significativamente más altos que los valores postratamiento (5,5 +

1,81 frente a 3,15 + 1,41, p <0,0001). Los evaluadores ciegos calificaron la mejora final de 2 pacientes como altamente satisfactoria, de 8 pacientes como satisfactoria, de 18 pacientes como levemente satisfactoria y de 3 pacientes como insatisfactoria.
Mediante la tinción de rojo Picrosirus se observó un aumento significativo en la proporción de colágeno (COL) III (nuevo COL inmaduro) entre los grupos de control, laser + NSS y laser + PRP (13,9 + 15,97 vs 24,17 + 21,67 vs 34,41 + 23,57, respectivamente, p <0,0001). Por otro lado, se observó una disminución significativa simultánea en el cociente COL III (antiguo COL maduro) entre los grupos de control, laser + NSS y laser + PRP (84,77 + 15,6 vs 75,05 + 21,05 vs 64,5 + 23,13, respectivamente, p <0,0001).

Conclusiones: Las combinaciones de AFL, NAFL y PRP para el tratamiento de cicatrices atróficas del acné producen mejoras significativas de forma temprana cuando se usan simultáneamente desde la primera sesión. Esta triple combinación mejoró la remodelación de la cicatriz, según la autoevaluación del paciente 4 semanas después de la primera sesión, con mínimos efectos adversos.

1. INTRODUCTION

1.1. Post-Acne Scars; Pathogenesis, Classification, Gradings And Treatment Options

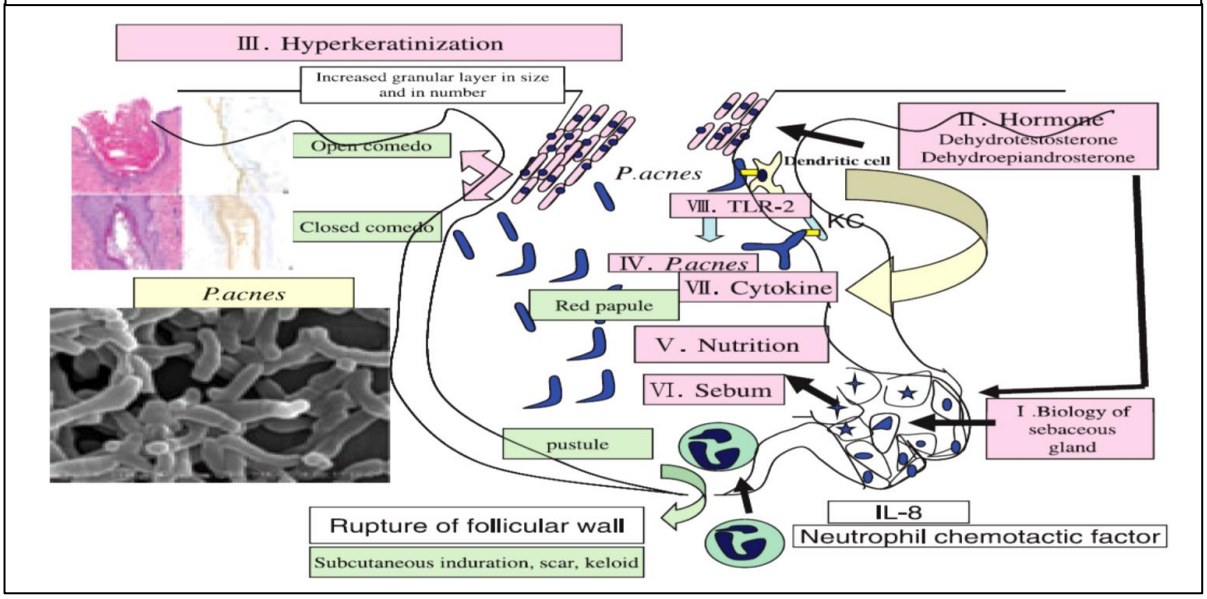
Introduction

1.1.1. Introduction

Acne has a prevalence of over 90% among adolescents and persists into adulthood in approximately 12%–14% of cases with psychological and social implications of high gravity.(1-3) All body areas with high concentrations of pilosebaceous glands are involved, but particularly the face, back, and chest. Inflammatory acne lesions can result in permanent scars, the severity of which may depend on delays in treating acne patients. This sequela is estimated to occur in up to 95 percent of acne patients and results in significant psychological distress for many individuals.(4)

Hormonal factors are involved in sebum excretion and hyperkeratinization in the infundibulum of pilosebaceous glands. Acne starts as microcomedones, caused by hyperkeratinization in the infundibulum, with increased size and number of granular layers. Microcomedones evolve either into open or closed comedones. The follicular channel is colonized by Propionibacterium acnes, which develops and stimulates cytokines production via toll-like receptor (TLR-2), resulting in inflammatory lesions. Nutrition may be involved in acne pathogenesis. IL-8, a neutrophil chemotactic factor, attracts neutrophils into the follicular walls as shown in Figure (1).(5)

Figure (1). Pathogenesis of acne. The biology of sebaceous gland (I) has been elucidated recently. Hormonal factors (II) are involved in sebum excretion (VI) and hyperkeratinisation in the infundibulum (III). Acne starts as microcomedones, which are generated by hyperkeratinisation in the infundibulum, with increased in size and in number of granular layers. Microcomedones evolve into open or closed comedones. The follicular channel is colonized by Propionibacterium acnes (IV), develops and stimulates cytokine production (VII) via toll-like receptor (VIII), resulting in inflammatory lesions. Nutrition (V) may be involved in acne pathogenesis. IL-8, a neutrophil chemotactic factor, attracts neutrophils into the follicular walls. Once the follicular walls rupture, granulomatous lesions with subcutaneous induration, scarring and keloids are generated. Adapted from (5).



1.1.2. Wound healing and scars pathogenesis

All healing scars will go through phases of healing including inflammation, granulation tissue formation, and extracellular matrix remodelling, as follows:

1.1.2.1. Inflammation phase: It lasts up to one week. This phase plays an important role in the development of post-acne erythema and hyperpigmentation in which melanogenesis may also be stimulated. In a study by Holland et al., biopsy specimens of acne lesions from the back of patients with severe scars and without scars were compared, they found that the inflammatory reaction at the pilosebaceous gland was stronger and had a longer duration in patients with scars versus those without; in addition, the inflammatory reaction was slower in those with scars versus patients who did not develop scars. The strong correlation of severity and duration of inflammation to the development of scarring emphasized that treating early inflammation in acne lesions may be the best approach to prevent post-acne scarring.(6)

1.1.2.2. Granulation Tissue Formation: In this phase, damaged tissues are repaired, and new capillaries are formed. Several growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factors (TGF-α and TGF-β), are released to stimulate the migration and proliferation of fibroblasts.(7) Fibroblasts start new COL production approximately after 3 to 5 days. Earlier, the new skin composition is dominated by type III COL, with a small percentage (20%) of type I COL. Later, the balance of COL types shifts in mature scars to be similar to that of unwounded skin, with approximately (80%) of type I COL.(8)

1.1.2.3. Extracellular Matrix Remodelling: Fibroblasts and keratinocytes produce enzymes including those that determine the architecture of the extracellular matrix metalloproteinases (MMPs) and Tissue Inhibitors of MMPs (TIMPs). MMPs are extracellular matrix (ECM) degrading enzymes that interact and form a lytic cascade for ECM remodeling.(9) Therefore, any imbalance in the ratio of MMPs to tissue inhibitors of MMPs could result in the development of atrophic or hypertrophic scars. Inadequate response results in diminished deposition of COL and formation of an atrophic scar, whereas, if the healing response is too exuberant, a raised nodule of fibrotic tissue forms hypertrophic scars.(10)

Acne lesions are unusual in that the inflammation is initiated beneath the epidermis in the infrainfundibular region of the pilosebaceous structure.(11) The follicular walls rupture through the weakened infrainfundibular section of the follicle. The end-stage result of follicular rupture is a perifollicular abscess. Small abscesses incorporating the horny core will discharge

through the skin. This will be repaired without scarring in about 7–10 days. The epidermis is always attempting repair, and cells grow from the epidermis and appendageal structures to encapsulate the inflammatory reaction. If this is complete, there is a resolution of the lesion without incident. Sometimes, however, this encapsulation is incomplete and further rupture occurs. If the dermal inflammation is severe, total necrosis of the follicle may ensue and sloughing will produce a focal scar. If the inflammation is much more severe, and especially if rupture occurs deeply in the follicle, the inflammation will extend well beyond the environment of the hair follicle into the subcutis, along vascular channels, and around sweat glands.(12)

Thus, the subsequent scarring often involves deeper structures rather than just the skin surface. As the scars mature and contract, they draw in these surface layers, leading to the appearance of indention or atrophy. The enzymatic activity and inflammatory mediators also destroy the deeper structures and this loss of deeper skin architecture adds to the production of atrophic scarring.(13)

Strikingly, the exact pathogenesis of atrophic acne scarring is not completely understood but is most likely related to inflammatory mediators and enzymatic degradation of COL fibers and subcutaneous fat. It is unclear why some acne patients develop scars while others do not, as the degree of acne does not always correlate with the incidence or severity of scarring. However, the scarring process can occur at any stage of acne; thus, it is uniformly believed that early intervention in inflammatory and nodulocystic acne is the most effective way of preventing post-acne scarring. Once scarring has occurred, it is usually permanent.(6, 14)

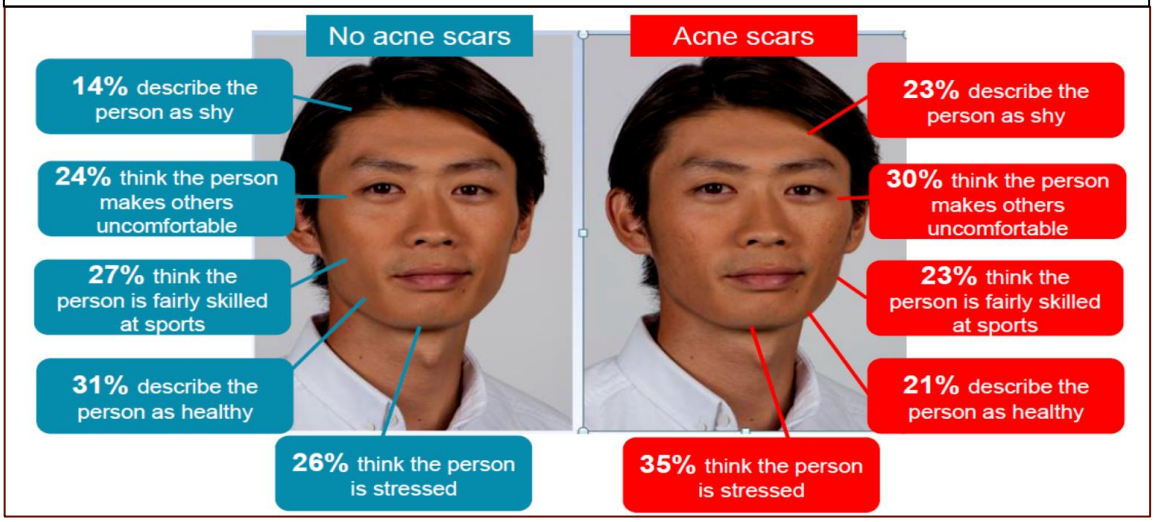
1.1.3. Psychosocial morbidity

The adverse psychological effects of acne and scarring sequelae have been identified for decades to be common because of the high prevalence of acne, especially among adolescents. The associated effects of acne and its complications that have been reported include poor self-esteem, anxiety, depression, suicidal ideation, social isolation, altered perception of body image and emotional instability. Moreover, Social morbidity associated with acne included decreased dating, decreased participation in sports, decreased eating out, impaired academic performance and increased unemployment.(6)

Recently, Dreno et al have performed an interesting online survey in 6 countries with 4618 respondents, presenting facial images of models with no scars and with digitally

superimposed acne scars. Scarred individuals were more likely to be categorized as shy or stressed, and less likely to be perceived as successful, happy, confident, healthy, reliable, or leader. The majority of adults (66%) affected by acne scars reported willingness to pay money to eradicate the blemishes as well as a greater likelihood of spending money on an effective treatment than on high-end apparel or shoes. They concluded that facial images with acne scarring engendered unfavourable initial impressions and were associated with negative

Figure (2): Presence of Post-acne has a negative impact on impressions. Adapted from (15)

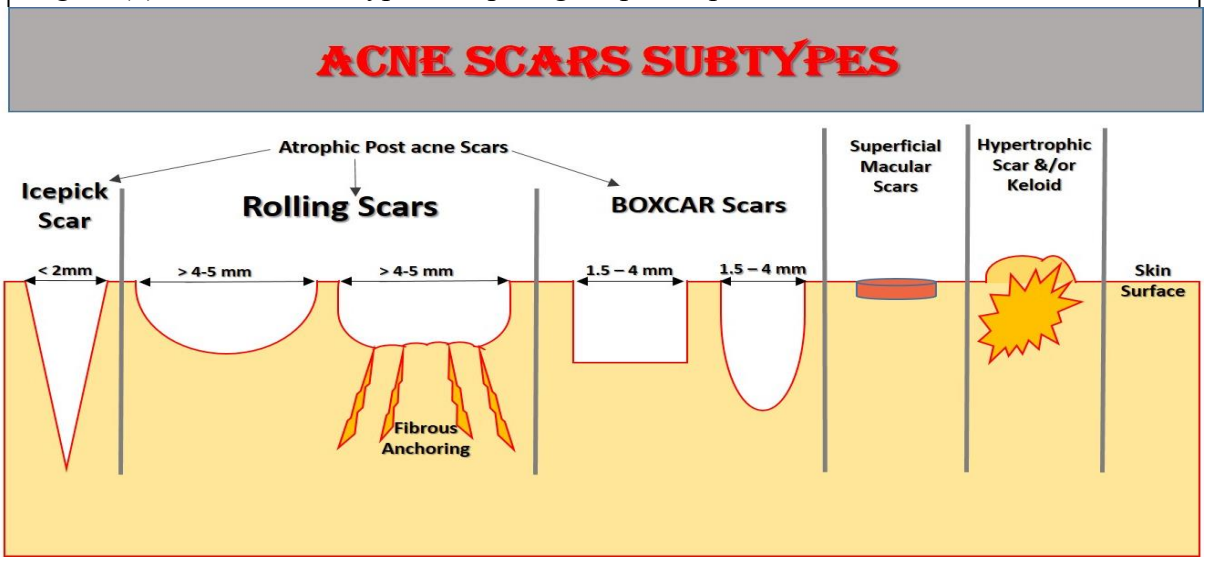


emotional and personal attributes.(15)

1.1.4. Classification of post-acne scars

Generally, there have been multiple classifications proposed by many authors. However, we would like to classify post-acne scars on basis of treatment approach into three

Figure (3): Acne Scar subtypes comparing shapes, depth, and width.



major different types: **(1)** Atrophic, **(2)** Superficial macular scars and **(3)** Hypertrophic &/or Keloidal scars as shown in Figure (3).

Goodman has proposed to identify two more types, perifollicular scarring and fat atrophy. Unfortunately, atrophic acne scars; which is the most difficult to treat, are by far the most common type and can be classified into 3 basic subtypes: (a) ice-pick scars, (b) rolling scars, and (c) boxcar scars.(13, 16)

1.1.4.1. Atrophic Scars: Atrophic acne scars are more common than keloids and hypertrophic scars with a ratio of 3:1. They have been subclassified into ice pick, boxcar, and rolling scars (Figure 3). With atrophic scars, the ice pick type represents 60%–70% of total scars, the boxcar 20%–30%, and rolling scars 15%–25%.(17)

a. **Icepick:** narrow (≤2 mm), punctiform, and deep scars are known as icepick scars. Usually, the opening is typically wider than the deeper infundibulum (forming a "**V**" shape).
b. **Rolling:** wider than 4 to 5 mm, characterized by dermal tethering of the dermis to the subcutis. Usually, these scars give an undulating appearance to the skin ("**M**" shape).
c. **Boxcar:** ranges from 1.5 to 4 mm in diameter, round or oval scars with well-established vertical edges. These scars tend to be wider at the surface than an icepick scar and do not have the tapering V shape. Instead, they can be visualized as a "**U**" shape with a wide base. Boxcar scars may be shallow or deep (Figure 3).

Clinically, the 3 different types of atrophic scars can be observed in the same patients and it can be very difficult to differentiate between them. For this reason, several classifications and scales have been proposed by other authors.(18) The following table summarizes the clinical features (Table 1).

Table (1): Acne Scar morphological description. Adapted from (17)

Acne Scars Subtype	Clinical Features
Icepick	Icepick scars are narrow (<2 mm), deep, sharply marginated epithelial tracts that extend vertically to the deep dermis or subcutaneous tissue.
Rolling	Rolling scars occur from dermal tethering of otherwise relatively normal-appearing skin and are usually wider than 4 to 5 mm. Abnormal fibrous anchoring of the dermis to the subcutis leads to superficial shadowing and a rolling or undulating appearance to the overlying skin.
Boxcar Shallow <3 mm diameter >3 mm diameter	Boxcar scars are round to oval depressions with sharply demarcated vertical edges, similar to varicella scars. They are clinically wider at the surface than icepick scars and do not taper to a point at the base.
Deep <3 mm diameter >3 mm diameter	They may be shallow (0.1–0.5 mm) or deep (≥0.5 mm) and are most often 1.5 to 4.0 mm in diameter.

1.1.4.2. Superficial Macular Scars: If only the epidermis and superficial dermis are involved, the scars may appear as discolored macules that may be either erythematous, if inflamed and comparatively early or young scars (under 1 year), or with altered pigmentation (Figure 3). Usually, such post-inflammatory hyperpigmentation will fade in 3–18 months if sun-protected and even topical treatment may not always be required. If topical treatment is needed, topical creams such as retinoic acid or alpha-hydroxy acids can be used in conjunction with topical steroids. Also, light skin peels with glycolic acids and/or Jessner's solution may be utilized.(13)

1.1.4.3. Hypertrophic &/or Keloidal Scars: Hypertrophic and keloidal scars are associated with excess COL deposition and decreased collagenase activity. Hypertrophic scars are clinically pink, raised, and firm lesions. Their histology is similar to that of other dermal scars with thick hyalinized COL bundles that remain within the borders of the original site of injury. On the other hand, keloids are presented clinically as reddish-purple papules and nodules that proliferate beyond the borders of the original wound; histologically, they are characterized by thick bundles of hyalinized acellular COL arranged in whorls. Hypertrophic and keloidal scars are more common in darker-skinned individuals occurring predominantly on the trunk.(18)

According to Goodman, perifollicular acne inflammation produces hypopigmented papular scars from the destruction and attenuation of COL and elastin fibers in the tissues around hair follicles most commonly on the trunk. While Lipoatrophy is seen after total resolution of space-occupying cystic acne lesions in the face, which leave a void that cannot be filled by the atrophied subcutaneous tissues. Thus, the skin surface is drawn deeper by the contracture of the scarring around these cysts.(13, 18)

1.1.5. Evaluation and grading of post-acne scars

Various attempts have been made to describe types of post-acne scars morphologically and suggest appropriate therapy. First, scar topography is usually the most important and variable characteristic. Second, patient characteristics such as skin type and amount of disease are also of importance. It is in vain to embark on the treatment of post-acne scarring unless knowledge is obtained from many of the described techniques because treating acne scarring is difficult and unrewarding if not optimally addressed. Therefore, each scar and each patient must be treated individually according to the patient and scar characteristics.(19)

Introduction

In 2006, Goodman and Baron proposed a quantitative global acne scar grading system for assessing load and global severity of the disease. Photographs of 21 patients were assessed independently by four observers, two of whom were physicians and the other two were nurses. No substantial difference among acne scarring scores was seen between observers, with inter-rater agreement within four score points in 19 of the 21 patient-photos assessed. They concluded that their reproducible grading system (Table 2) would allow investigators and proceduralists independent of medical background, to compare their cases more accurately and to have a more objective discussion of the efficacy of operative interventions or therapies.(20)

Table (2): Quantitative Global Acne Scar scoring. Adapted from (20)

(Grade) Type	Number of lesions: 1 (1–10)	Number of lesions: 2 (11–20)	Number of lesions: 3 (> 20)
(A) Milder scarring (1 point each) Macular erythematous or pigmented Mildly atrophic dish-like	1 point	2 points	3 points
(B) Moderate scarring (2 points each) Moderately atrophic dish-like Punched out with shallow bases small scars (< 5 mm) Shallow but broad atrophic areas	2 points	4 points	6 points
(C) Severe scarring (3 points each) Punched out with deep but normal bases, small scars (< 5 mm) Punched out with deep abnormal bases, small scars (< 5 mm) Linear or troughed dermal scarring Deep, broad atrophic areas	3 points	6 points	9 points
(D) Hyperplastic Papular scars	2 points	4 points	6 points
(D) Hyperplastic Keloidal/hypertrophic scars	Area < 5 cm^2 6 points	Area 5–20 cm^2 12 points	Area > 20 cm^2 18 points

This quantitative system described a numerical scoring system of post-acne scars severity, both atrophic and hypertrophic, the numerical total being tallied from the number and severity of the different types of scars seen by the observer. Although it is accurate with a numerical score to the patient, however, it is somewhat cumbersome for daily use.(21)

Amazingly, few months later, the same authors proposed a qualitative global acne scar grading system. This was different from the classification of individual scar morphology which may give an indication of the inducing pathophysiology and hint at the required treatment for that scar type but does not describe the patient's disease load or global severity. Due to the pleomorphism of post-acne scars, they are difficult to count, and they are equally difficult to photograph because of their three-dimensional nature.(21) Actually, this qualitative global grading came a long time after the consensus conference on acne classification held in Washington, DC, in 1990. Patients differ considerably in their abilities to withstand the psychological, social, and occupational effects of both acne and its consequent scarring. Thus,

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Pochi et al on their report of the consensus conference felt that pattern-diagnosis, including a global evaluation of lesions, would best consider the ‘‘total impact of the disease”.(22)

Hence, the aim of Goodman’s qualitative classification was to establish an index of severity of an individual’s condition that can be readily acknowledged, recorded, and compared over time or at a point in time in a clinic or between different clinics. In this classification (Table 3), four grades of scars were differentiated and may be further subdivided by focal area of involvement.(21) This was a great step towards facilitating the relatively simple grading of a patient with post-acne scarring and allow the rational description of that patient.

Table (**3**): Qualitative Global Acne Scar scoring. Adapted from (21)

Grade	*Level of disease*	*Characteristics*	*Examples of scars*
1	Macular disease	Erythematous, hyper- or hypopigmented flat marks visible to patient or observer irrespective of distance.	Erythematous, hyper- or hypopigmented flat marks
2	Mild disease	Mild atrophy or hypertrophy that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in males or normal body hair if extrafacial.	Mild rolling, small soft papular
3	Moderate disease	Moderate atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair if extrafacial, but is still able to be flattened by manual stretching of the skin.	More significant rolling, shallow ‘‘box car,’’ mild to moderate hypertrophic or papular scars
4	Severe disease	Severe atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair (if extrafacial) and is not able to be flattened by manual stretching of the skin.	Punched out atrophic (deep ‘‘box car’’), ‘‘ice pick’’, bridges and tunnels, gross atrophy, dystrophic scars significant hypertrophy or keloid

Introduction

Moreover, in 2007, another grading scale named ECCA (échelle d'évaluation clinique des cicatrices d'acné) was published by Dreno et al. The authors developed it in the form of 6 items, as shown in (Figure 4), designed to assess easily and quickly the severity of acne scars by a global score though calculations in (Table 4). The interobserver reliability of the ECCA grading was statistically validated by showing the reliability of this scale among 7 dermatologists who used it on the same group of 10 acne patients. Their statistical analysis confirmed that there was no effect of dermatologists on the global grading (p= 0.18) or on the subgradings 1 and 2 (p= 0.31 and p= 1, respectively), which allows them to conclude that the 7 dermatologists scored the 10 acne patients homogeneously. Therefore, they considered ECCA grading scale a reliable assessment of the acne scars after a short training on how to use it.(23)

Figure (4): Acne scars assessed by the ECCA grading scale. Adapted from (23)

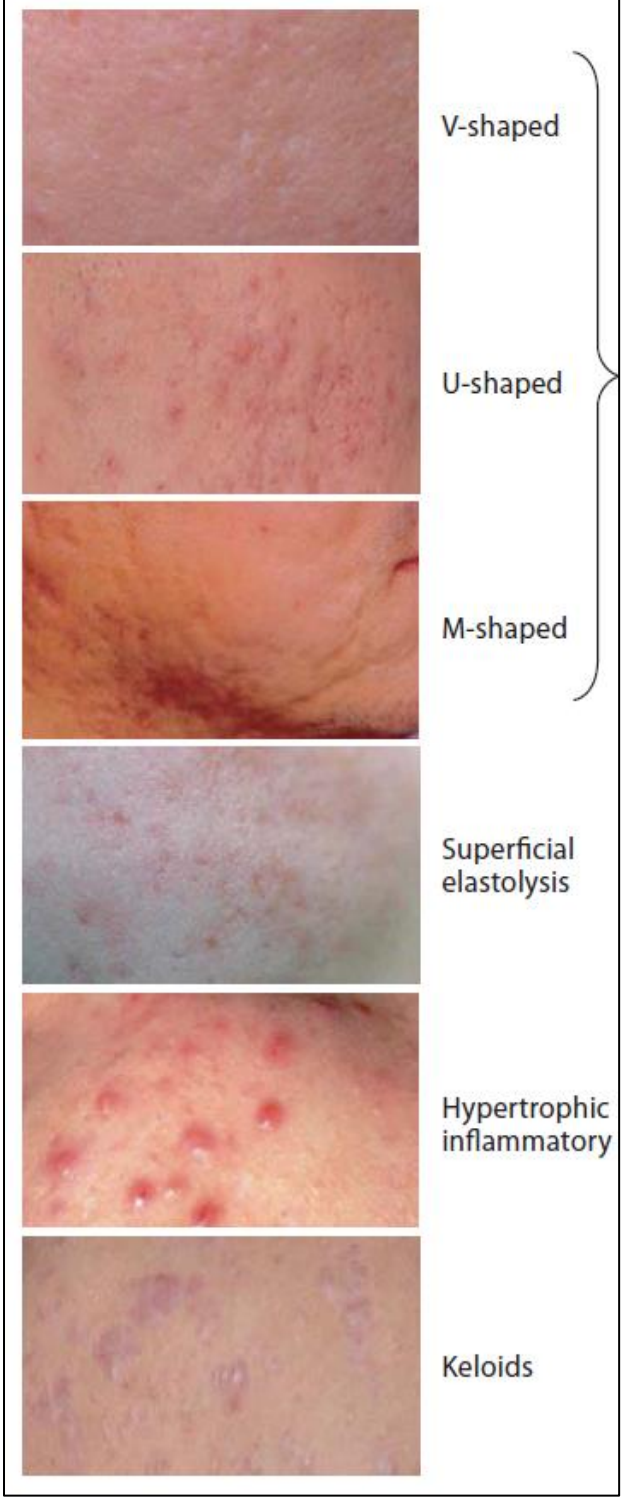


Table (4): ECCA grading scale. Adapted from (23)

Description	Weighting factor (a)	Semi-quantitative score (b)	Grading (a × b)
V-shaped atrophic scars, diameter of less than 2 mm, and punctiform	15	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
U-shaped atrophic scars, diameter of 2–4 mm, with sheer edges	20	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
M-shaped atrophic scars, diameter of more than 4 mm, superficial and with irregular surface	25	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Superficial elastolysis	30	0 = absent 1 = mild 2 = moderate 3 = intense	/_____/
Subgrading 1			/_____/
Hypertrophic inflammatory scars, scars of less than 2 years of age	40	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Keloid scars, hypertrophic scars, of more than 2 years of age	50	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Subgrading 2			/_____/
Global score (subgradings 1 + 2)			/_____/

As shown in the table above, subgrading 1 is composed of the grading of the first 4 items, whereas subgrading 2 is composed of the grading of the last 2 items. Therefore, the global score could vary from 0 to 540 depending on the severity of the scars in terms of their nature and number.

However, since 2007, the ECCA grading scale was not widely used in publications. Only 3 publications were found to make use of such a helpful tool.(24-26) This may be due to that researchers found it time-consuming or a sophisticated tool in need of training and practice. On the contrary, the qualitative global acne scar grading proposed by Goodman et al was much more widely used.

Introduction

In 2010, Tan et al proposed another Global Scale for Acne Scar Severity (SCAR-S) including a six-category global system ranging from clear to extremely severe (Table 5) and based on a global evaluation scale modified for acne scarring in a large cohort study including 973 acne patients. In conclusion, they considered SCAR-S a practical, validated, global system clinically relevant and related with acne duration, and suitable for overall severity grading.(27)

Table (5): Global Scale for Acne Scar Severity. Adapted from (27)

Category	*Score*	*Description*
Clear	0	No visible scars from acne
Almost clear	1	Hardly visible scars from 2.5 m away
Mild	2	Easily recognizable; less than half the affected area (eg, face, back, or chest) involved
Moderate	3	More than half the affected area (eg, face, back, or chest) involved
Severe	4	Entire area involved
Very severe	5	Entire area with prominent atrophic or hypertrophic scars

In 2013, Lacarrubba et al proposed a new technique for qualitative evaluation of post-acne scars by a non-invasive High-frequency ultrasound correlation to clinical evaluation. In eighty-one lesions, they found out that ultrasound results generally correlated with clinical appearance, although that eight clinically ice pick scars showed a typical boxcar morphology upon ultrasound examination. In their study, the ultrasound showed that ice pick and boxcar scars were the deepest, with 95.8% between 0.2 and 0.5 mm.(28)

Recently, in 2016, Layton et al developed new patient-oriented tools for assessing atrophic acne scars by using two patient-reported measures, the Self-assessment of Clinical Acne-Related Scars (SCARS) and the Facial Acne Scar quality of Life (FASQoL) tools. Both tools were developed according to Food and Drug Administration (FDA) guidance methodology specifically to help clinicians assess the severity and impact of facial atrophic acne. The authors focus on symptoms (SCARS), psychological and social well-being (FASQoL), and designed their tools to be suitable for self-completion and to be rapidly completed (2–5 min) within clinical research setting.(29)

SCARS tool was designed as two Visual Analog Score (VAS) and a 5-item questionnaire (Table 9), while FASQoL tool was a 10-item questionnaire assessing atrophic acne scar-related impacts (Table 10). Most concepts were relevant and understood by tested patients; items were worded so that subjects could provide meaningful responses.(29)

1.1.6. Treatment options of atrophic post-acne scars:

Treatment of acne scarring is paramount due to its impact on self-esteem, social interactions, and even the ability to obtain employment.(30) Several treatments are available to reduce the appearance of scars. Treatment of acne scars must be individually directed for each patient depending on the types of scars present. The following; as shown in (Table 6), is an updated classification for the procedures used for post-acne scars management:

Table (6): Treatment Options:

1.1.6.1. Resurfacing Procedures:

- **1.1.6.1.1. Chemical Peels**
- **1.1.6.1.2. Dermabrasion**
- **1.1.6.1.3. LASER Resurfacing**

1.1.6.2. Lifting Procedures:

- **1.1.6.2.1. Subcision**
- **1.1.6.2.2. Fillers**
- **1.1.6.2.3. Platelets Rich Plasma (PRP)**
- **1.1.6.2.4. Autologous Fat Transfer (AFT)**
- **1.1.6.2.5. Stem Cells (SC)**
- **1.1.6.2.6. Punch Elevation**

1.1.6.3. Excision Procedures:

- **1.1.6.3.1. Punch Excision**
- **1.1.6.3.2. Elliptical Excision**
- **1.1.6.3.3. Punch grafting**

1.1.6.4. Other Procedures:

- **1.1.6.4.1. Skin Needling**
- **1.1.6.4.2. Nonablative Radiofrequency**
- **1.1.6.4.3. Microneedling Radiofrequency**

1.1.6.5. Combination Techniques for 2 or more procedures

1.1.6.1. *Resurfacing procedures:*

They act by removing layers of skin from the top down. Injury to the dermis by resurfacing procedures is thought to cause dermal remodeling and neocollagenesis.

1.1.6.1.1. Chemical Peels:

Medium-depth peels, such as trichloroacetic acid (TCA) have shown varying results for acne scars but are limited by their unpredictable degree of penetration beyond the papillary dermis. In a study of 15 patients receiving 1 to 3 peels consisting of Jessner's solution followed by 35% TCA for ice-pick scarring, at least some improvement was observed in 14 of 15 patients; however, significant improvement was seen in only one patient. Furthermore, 73.4 % of patients experienced post-inflammatory hyperpigmentation, which lasted up to three months in some individuals.(31) Deeper peels, such as phenol, can also treat acne scars. In one study, 7 out of 11 patients achieved more than 50-percent improvement. However, significant side effects, such as scar formation and hypopigmentation, persisted beyond six months.(32)

However, Lee et al reported a new technique using focal application of TCA which they called the **C**hemical **R**econstruction of **S**kin **S**cars (**CROSS**) method. This technique was designed to take advantage of the dermal thickening and COL production that occurs when higher concentrations of TCA are applied while minimizing such side effects as scarring and dyspigmentation. TCA is applied to the epithelial lining of the scar until frosting occurs.(33)

1.1.6.1.2. Dermabrasion:

Dermabrasion was the first major advance in the treatment of acne scars since the 1950s via a serrated wheel, diamond embedded fraises, sterilized sandpaper or wire brush attached to a rapidly rotating handpiece that evenly abrades the skin to the papillary dermis. In contrast, microdermabrasion utilizes aluminium oxide crystals delivered through a nozzle to superficially abrade the stratum corneum through a series of micro lacerations.

Although it may be particularly helpful in softening sharper scar edges, the technique is highly operator dependent, with errors resulting in significant scarring, postoperative pain and healing times up to one month, with the tendency to form milia.(13) Because of these disadvantages, dermabrasion has largely been replaced by resurfacing lasers.

1.1.6.1.2. Laser Resurfacing:

Over the past decade, laser resurfacing has emerged at the forefront of acne scar treatment. The first lasers to be used for acne scarring were the ablative CO_2 and Er: YAG lasers, which emit radiation at wavelengths of 10,600 and 2,940 nm, respectively, targeting water in the epidermis to stimulate COL synthesis. In 1996, Alster et al published a large study on the use of ablative CO_2 for acne scarring, showing a mean improvement of 81.4 percent in 50 patients with moderate-to-severe acne scars.(34) It should be noted that in the authors' experience, such high rates of improvement are not always observed. On the other hand, response rates to the original short-pulse Er: YAG lasers ranged from 25 to 90 percent, with the largest study of 21 patients reporting mean improvement in acne scarring of 40 percent.(35) Despite the results with these lasers, adverse events, such as post-inflammatory hyperpigmentation and prolonged erythema were pronounced. Also, more serious complications, including infection and scarring, have been reported.(36)

Following the advent of ablative lasers, efforts were made to develop devices with

a better safety profile, leading to the introduction of the nonablative, long-pulsed 1450 nm diode and 1320 nm Nd: YAG lasers. These mid-infrared wavelengths target water in the dermis to stimulate COL synthesis. Both lasers showed modest efficacy after 3 to 6 treatments in improvement of acne scarring.(37-39) In a prospective, split-face comparison of 20 patients with atrophic acne scarring treated with the nonablative long-pulsed 1450 nm diode and 1320 nm Nd:YAG, all patients demonstrated mild improvement after three treatment sessions, with a trend toward greater scar improvement in the 1450 nm diode group.(40) Although side effects were minimal with the nonablative lasers, their efficacy did not compare to traditional ablative laser therapy.

Meanwhile, in 2004, the Fractional Photothermolysis (FP) theory revolutionized acne scar treatment and the first fractional laser (Fraxel, Solta Medical, Mountain View, California), a 1550 nm erbium-doped laser, was introduced.(41) A series of five treatments for 17 acne patients with ice-pick, boxcar, and rolling scars were examined. Mean clinical improvement ranged from 25 to 50 percent using digital photography and from 22 to 66 percent using typographic imaging. Side effects were limited to temporary postprocedure erythema and edema.(42) In 2007, a second-generation erbium-doped 1550 nm laser (Fraxel SR1500) was approved by the FDA, which delivers a higher pulse energy of up to 70 mJ, resulting in deeper tissue penetration. Most of the studies on Non-Ablative Fractional Laser Resurfacing (**NAFL**) included patients with Fitzpatrick skin type IV and V, with no post-inflammatory hyperpigmentation (PIH) observed. However, a more recent study found that even at energies as low as 10 mJ, PIH can occur.(43) Thus, caution should still be exercised when treating darker-skinned individuals with NAFL.

As technology has advanced, ablative fractionated CO2 and erbium lasers have also been developed to achieve more prolonged COL remodeling. The effectiveness of Ablative Fractional Laser Resurfacing (**AFL**) was first demonstrated by Chapas et al, in which 13 patients with acne scarring received 2 or 3 monthly treatments with fractional CO2 (Fraxel Re:pair Laser Prototype, Solta Medical, Mountain View, California), resulting in a mean scar depth improvement by topographic analysis of 66.8 %. Side effects included post-procedure erythema, edema, and petechiae, which resolved by seven days. Unlike traditional ablative resurfacing, no delayed onset pigmentary changes were observed.(44) A study by Kim et al, examining AFL at low energy followed by nonablative 1064nm Nd:YAG, reported that clinical efficacy was better than that of AFL alone at higher energy, with fewer adverse events.(45) The benefits of AFL are that it more closely rivals the efficacy of traditional ablative laser therapy than does NAFL yet

without the long downtime and risk of permanent scarring or dyspigmentation seen with ablative lasers.(46)

1.1.6.2. ***Lifting procedures:*** They act by drawing the base of a deep scar upward towards the surface and making the skin surface smoother.

1.1.6.2.1. Subcision:

It was first introduced in 1995 as an effective treatment for rolling scars.8 In this procedure, a hypodermic, tribevelled, or filter needle is introduced into the subdermal plane to undermine the scar through a series of backward and forward motions, followed by horizontally rotating the needle in a fanning manner.(14) These motions loosen the fibrotic adhesions that cause the bound-down appearance of rolling scars and create a wound environment amenable to COL deposition. The bleeding and subsequent clot formation resulting from the procedure aid in the skin release and elevation from the underlying scar tissue, generating a potential space for neocollagenesis. In a study of 40 patients undergoing subcision for rolling scars, the overall degree of improvement was rated 51 percent by patients and 50 to 60 percent by investigators.(47) However, patients in other studies have developed hypertrophic scarring requiring treatment with intralesional steroids.(13)

1.1.6.2.2. Fillers:

Tissue augmentation is aimed at replacing tissue volume as well as stimulating COL production by native fibroblasts in acne scars.(48) Recently, COL fillers have been replaced by products with less allergenic potential, including hyaluronic acid (HA), calcium hydroxyapatite, poly-L-lactic acid (PLLA) and polymethylmethacrylate. Typically, dermal fillers are reserved for larger, rolling scars given the size of the injected molecules and the degree of precision required for delivery.

Although HA fillers are commonly used for scars, there is little data in the literature regarding the use of fillers specifically for acne scars. Recently, a new technique known as subdermal minimal surgery (Airgent, PerfAction, Inc., Rehovot, Israel) has been developed in which a needleless hypodermic inoculator delivers HA through a high-pressure jet, allowing for more precise and even radial dispersion into the dermal planes. In a study of 10 patients, eight had at least 50-percent improvement in acne scar appearance.(49)

Also, Calcium hydroxyapatite (Radiesse, Merz Aesthetics) is a semi-permanent filler that has been shown to improve rolling scars, but not ice-pick scars. Goldberg et al demonstrated improvement in ten patients after just one treatment maintained for up to 12 months.(50)

1.1.6.2.3. Platelets Rich Plasma (PRP):

Autologous PRP can enhance wound healing by accelerating tissue repair and reducing postoperative pain.(51, 52)Their effect in acne scarring was first noted during intradermal injections of PRP for skin rejuvenation.(53) This was due to their mediator role in tissue repair through the release of growth factors, cytokines, and chemokines from their α-granules.(54) Topical PRP has a synergistic effect with skin needling in atrophic acne scars, as the skin needling creates a way for PRP absorption to occur and allows the additional platelets to contribute to wound healing.(18, 55) Furthermore, intradermal injections and/or topical application of PRP after fractional ablative CO_2 (10,600 nm) or Er:YAG (2,940 nm) lasers enhanced the recovery of laser damaged skin and improved the clinical appearance of acne scars compared to control.(56-59) In 2019, PRP combination with Subcision has been repeatedly investigated with favourable outcomes regarding improvement, tolerability, and safety with fewer complications.(60-63)

1.1.6.2.4. Autologous Fat Transfer (AFT):

Acne scars often subcised immediately prior to treatment with AFT. In a comparative study comparing three sessions of fractional CO2 laser in 10 patients to one session of AFT in another 10 acne scar patients, AFT proved more effective after a short follow up of 3 months. In the fractional CO_2 laser group, under 20% of patients were graded as having excellent scar improvement, 0 as having marked scar improvement, under 10% as having mild scar improvement, and almost 70% as having moderate scar improvement. In the AFT group, the scar and overall improvement were graded as 30% excellent, 30% marked, 20% moderate, and 20% mild.(64) On the other hand, a single earlier long term follow-up study in 2000 has revealed a limited duration of AFT effect when used for other applications. Thus, although AFT may be effective for acne scarring, results are not permanent and the procedure is highly operator- dependent.(65) However, many who originally reported failure in various applications eventually report success after altering their methods of harvesting, refinement, and placement. Early research has indicated the possible involvement of more undifferentiated cells in some of the observed

effects of fat grafting on surrounding tissues. Of particular interest is the research that has pointed to the use of stem cells to repair and become bone, cartilage, muscle, blood vessels, nerves, and skin. Further studies are essential to understand grafted fat tissue.(66)

1.1.6.2.5. Stem Cell (SC):

Friedenstein and colleagues identified the first population of Mesenchymal Stem Cells (MSCs) in the bone marrow stroma, in the late 1960s. They demonstrated that these cells could self-renew and differentiate into osteoblasts, chondrocytes, adipocytes, and hematopoietic supporting stroma by transplanting in vivo an MSC suspension derived from a single cell expansion.(67)

In 2014, a pilot study has been conducted in 14 patients with moderate-to-severe facial atrophic acne scars. Autologous bone marrow Stem cell (BMSCs) aspiration was preceded by administration of granulocyte-colony stimulating factor (G-CSF). The solution containing the stem cells was injected under each scar intradermally. Qualitative and quantitative evaluations conducted 6 months after the procedure found significant improvement in the appearance of the scars and no reported adverse events.(68)

On the other hand in 2013, Chavez-Munoz et al have demonstrated for the first time that Adipose-derived Stem Cells (ASCs) have the capacity to transdifferentiate into keratinocyte-like cells (KLCs) and an engineered stratified epidermis.(69) It is important to highlight that the isolation of ASC yields more stem cells per gram of tissue than from bone marrow.(70, 71) Moreover, the easier way of aspiration by either enzymatic or non-enzymatic methods made it accessible for plastic or dermatologic surgeons to apply in different indications. Thus, a door was open for further studies in aesthetic fields.

In 2016, Zhou et al conducted a split-face study using conditioned media of ASCs combined with ablative CO_2 fractional resurfacing for 13 atrophic acne scars patients and 10 skin rejuvenation patients. Three sessions of Fractional CO_2 on both face sides combined with ASCs on a randomly selected face side were performed. Biopsies were taken from only one skin rejuvenation patient and analysed using haematoxylin and eosin, Masson's Trichrome, and Gomori's aldehyde fuchsin staining. In conclusion, they found that ASCs combined with laser increased subject satisfaction, elasticity, skin hydration, and skin elasticity and decreased Transepidermal Water Loss (TEWL), roughness, and the melanin index in both acne scars and skin rejuvenation groups. Histologic analysis showed that ASCs increased dermal COL density, elastin density, and arranged them in order.(72)

Recently, another split-face study was conducted for the evaluation of autologous ASCs vs. fractional CO_2 laser in the treatment of post-acne scars. In 10 patients, one side received ASCs single injection while the other received three sessions of Fractional CO_2. Scars were then assessed using the global scoring system Goodman and Baron and scar area percent using NIH ImageJ software. Both sides were followed for three months. The authors reported a significant improvement in the degree of scar severity, scar area percent, skin hydration, and TEWL after 3 months of treatment on both sides of the face with insignificant differences between both treatment modalities.(73)

1.1.2.6.6. Punch elevation:

Punch elevation is a variation of other punch techniques except that the punched-out scar is not discarded. The tissue cylinder is incised down to the level of the subcutaneous fat. The scar is left to float up to the same level as the surrounding skin. If it does not rise easily it may be transected free at the level of the fat. The cylinder of tissue will fix in place by the patient's serum and left to secondarily heal like a graft, held in position by a little surgical tape. Resurfacing can be performed 4–8 weeks later if required. However, this is quite a limited technique, relying on adequate texture and color of the scar's floor, which is an infrequent occurrence.(13)

1.1.6.3. ***Excisional Procedures:***

1.1.6.3.1. Punch excision:

Punch excision removes a pitted scar with a straight-walled disposable or hair transplant punch that is slightly larger than the scar being addressed. Grevelink and White have conducted a study on 21 patients (skin types I-II) using a combination of punch excision and traditional laser skin resurfacing. They reported a range of clinical improvement by the independent assessor of 25-50% in skin type 1,50-75% in skin type II-III. There was a patient subjective improvement of 25-50 % for skin type I, 50-75 % for skin type II and 75- 100 % for skin type III. There was no wound dehiscence, evidence of infection, or hypertrophic scarring of treated areas noted on follow-up. However, Postoperative hyperpigmentation was noted in five patients.(74)

1.1.6.3.2. Elliptical excision:

Spira has proposed elliptical excisions as management for scars that are larger than 3.5mm.(75)

1.1.6.3.3. Punch grafting:

The scar is first excised and discarded as with punch excision; in its place, a full-thickness skin graft is positioned, taken from an inconspicuous site, such as the postauricular scalp. Studies on the effectiveness of punch techniques are largely limited to small case reports involving few patients. In these reports, the grafts were placed slightly elevated above the surrounding skin, with dermabrasion performed 4 to 6 weeks later to correct any residual surface abnormalities. One disadvantage of this procedure is that it is often a painstakingly slow process. Complications may also arise, including graft depression, failure of the graft to take, or formation of sinus tracts.(76, 77) It should be noted that such a treatment modality when combined with other modalities, would allow for the treatment of deep ice-pick scars so that less aggressive resurfacing may subsequently be performed to achieve optimal cosmetic results.

1.1.6.4. ***Other Procedures:***

1.1.6.4.1. Skin needling:

Skin needling, also called "COL induction therapy" or "needle dermabrasion" is the technique of rolling a device composed of a barrel studded with hundreds of needles, which create thousands of micro punctures in the skin to the level of the papillary to mid-dermis. The optimal scars to treat with skin lesions are the same as fractional laser resurfacing— rolling acne scars, superficial boxcar scars, or erythematous or hypopigmented macular scars. The proposed mechanism by which skin needling improves acne scars is as follows: The dermal vessels are wounded, causing a cascade of events including platelet aggregation, release of inflammatory mediators, neutrophil, monocyte, and fibroblast migration, production, and modulation of the extracellular matrix, COL production, and prolonged tissue modulation.(78)

A sterile rolling device with needles of length 1.5-2.5 mm is rolled across the skin with pressure in multiple directions until the area demonstrates uniform pinpoint bleeding through thousands of micro puncture sites. Another study describes rolling the device four times in four different directions (horizontally, vertically, and diagonally right and left) for a total of 16 passes. In the author's experience, the number of passes required to achieve uniform pinpoint bleeding of the treatment area is variable and is inversely proportional to the density of the needles on the rolling barrel. After the procedure, an occlusive ointment is applied. Generally, the skin oozes for less than 24 hours and then

remains erythematous and edematous for 2 to 3 days. Usually, three or more treatments are required to achieve optimal clinical benefit, separated by four-week intervals.(79)

1.1.6.4.2. Nonablative Radiofrequency:

It can be used as a monotherapy or adjuvant therapy with fractional lasers. Radiofrequency (RF) delivers a current through the dermis that stimulates dermal remodelling, producing new COL and softening scar defects.(80) With traditional unipolar or monopolar RF, a single electrode allows for penetration deep into the dermis, but this is associated with increased pain and discomfort.(81) Newer Bipolar RF developments have allowed for more precision in the delivery of RF energy to the deeper tissues in the dermis, with decreased injury to the overlying epidermis.

1.1.6.4.3. Microneedling Radiofrequency:

Fractional RF uses an array of electrodes to create zones of thermal wounds that stimulate dermal remodelling. Microneedles can be used to deliver the electrical current to a specified depth within the dermis. Microneedle bipolar RF and fractional bipolar RF treatments offer the best results for acne scarring, particularly ice-pick and boxcar scars. Usually, an improvement of 25 to 75 percent can be expected after 3 to 4 treatment sessions. Such results are optimal three months after the final treatment due to the time required for fibroblast activation and upregulation of COL production. The adverse reactions associated with RF include transient pain, erythema, and scabbing that resolve within 3 to 5 days.(80, 82)

1.1.6.5. ***Combination of 2 or more procedures:***

The concept beyond the combination of procedures has strongly arisen in the last decade. Due to the variability in shapes, depth, and width of Post-cane scars (Figure **3**) that is usually seen in each patient, it is logical to apply combinations to achieve earlier improvement motivating the patient to continue the journey of consecutive sessions.

Dot peeling by CROSS is effective but has limitations in improving the texture of the skin. Fractional lasers have the advantage of improving skin texture and treating shallow and small atrophic scars but have limitations in affecting ice-pick and boxcar scars. Subcision effectively treats deep and wide depressed boxcar or rolling scars. PRP has a synergistic when combined with lasers and/or Subcision.

Thus, tailoring a combination of treatment procedures for each patient and early significant improvement is highly recommended.

1.2. Role of Fractional Lasers in Atrophic Post-Acne Scars

Introduction

1.2.1. Introduction to Selective Photothermolysis

Laser light can produce photothermal, photomechanical and photochemical reactions in the skin. Photothermal interactions are a result of heat generated by the laser light. If the skin is heated to a temperature below 50° C, then the consequent thermal tissue damage is reversible. At higher temperatures (50° -100° C), the coagulation of proteins occurs, leading to irreversible thermal tissue damage as shown in Figure (5). At temperatures above 100° C, the vaporization of tissue takes place. If the exposure time is shorter than the thermal relaxation time of the target (defined as the time required for a target to cool from the temperature that is achieved immediately after laser irradiation to half of that temperature), then the heat will not be able to diffuse, which limits the thermal damage to the target site. Tissue damage can also be limited to the target site using a laser with a wavelength that is specifically absorbed by the target tissue, where it is converted to heat and results in thermal injury. In addition to melanin, the skin chromophores that are important in laser surgery are haemoglobin, water and exogenous pigments such as tattoos. Carbon dioxide and Er: YAG lasers can be used as resurfacing devices because of their non-selective absorption by water. While, Yellow-light lasers, such as the pulsed-dye laser (PDL), are absorbed by haemoglobin and can be used for the treatment of vascular lesions.(83)

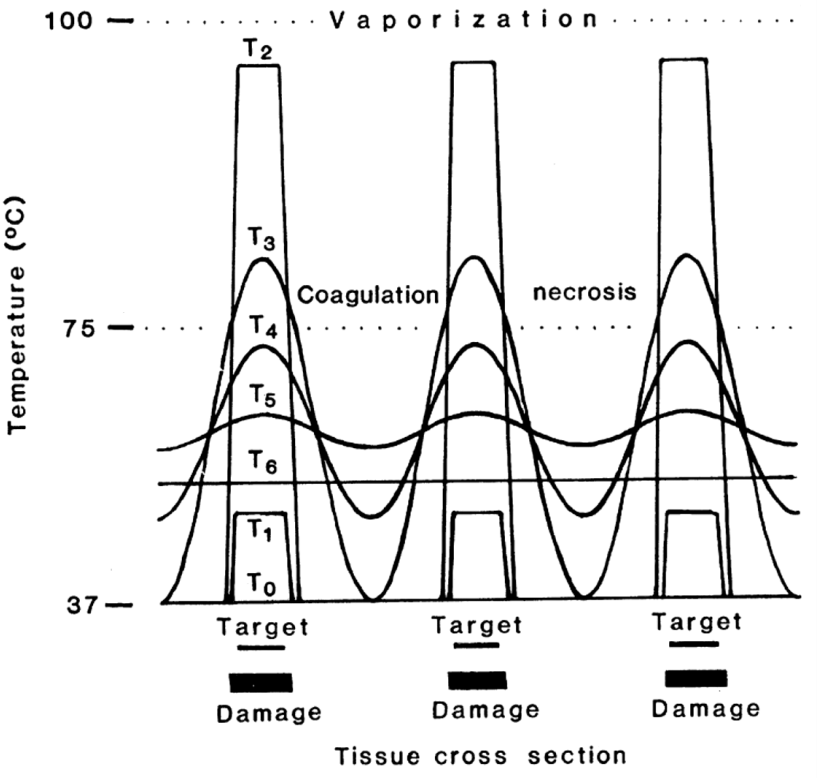


Figure (5): Schematic temperature profiles during selective Photothermolysis: T0 before laser exposure; T1 during laser exposure (selective rapid target heating); T2 at the end of laser exposure (targets irreversibly damaged); T3 one TRT after laser pulse (targets cooling, surrounding tissue warming); T4 Two TRT after laser pulse; T5 five TRT after laser pulse; and T6 tissue slowly returning to ambient thermal equilibrium.

*TRT= Thermal Relaxation Time. Adapted from (83)

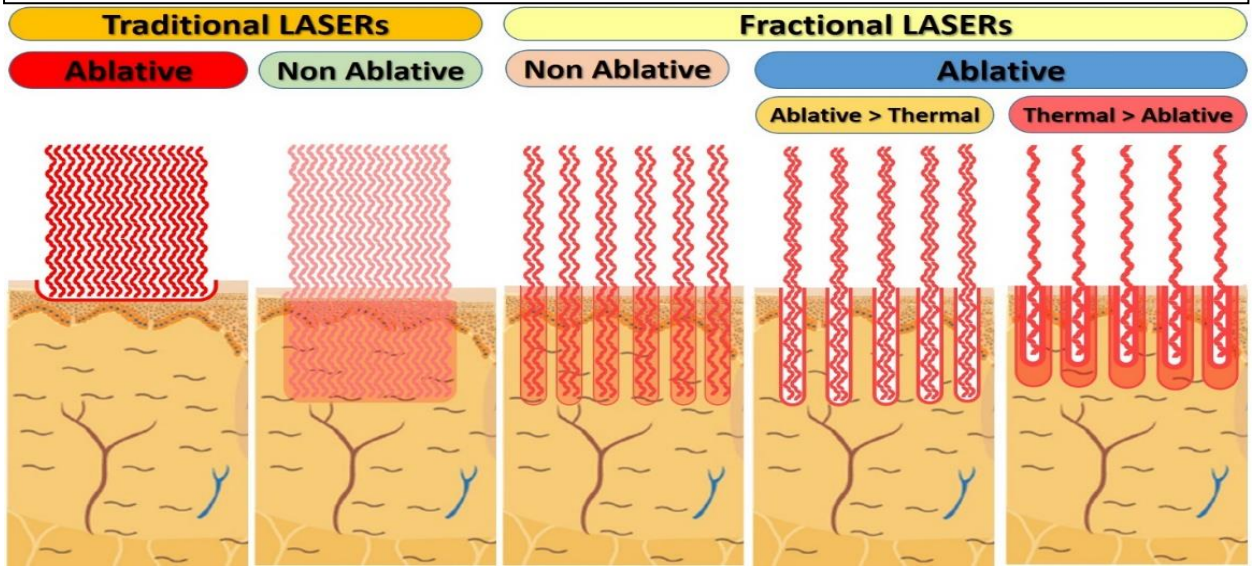
Introduction

In 1983, Anderson and Parrish proposed the theory of 'selective photothermolysis', in which selective tissue injury was achieved using high-energy, pulsed lasers. This theory revolutionized the dermatological use of laser systems, which have now become an important therapeutic option in the management of many skin conditions. The interaction of light with skin is determined by the optical properties of the skin components and the wavelength of the incident light. Selective tissue damage can be achieved using laser light with wavelengths that match those of the skin chromophores.(83)

In the early new millennium, a laser skin resurfacing renaissance occurred with the development of fractionated laser technology with its unique ability to produce Microscopic Thermal Zones (**MTZs**) of injury in the skin that enabled rapid healing without sacrificing clinical effect. Fractionated lasers have now become the mainstay of skin resurfacing treatment with excellent cosmetic outcomes and low-risk profile.(84)

Unlike fully ablative lasers, fractional lasers only treat a fraction of the skin. The percentage of the treated skin's surface typically varies from 2% to 95% depending on the device and number of passes. Also, the depth of the MTZ and the ablative vs. thermal effects of laser injury can be controlled by the physician (Figure 6).

Figure (6): Comparison among different resurfacing LASERs effect on Skin .



MTZs are separated by areas of normal skin, which acts as a reservoir for tissue regeneration and remodeling. These zones comprise up to 15 to 25 percent of the skin surface area per treatment session. Fractional lasers can be further subdivided into Ablative Fractional Lasers (**AFL**) and Non-Ablative Fractional Lasers (**NAFL**) depending on their impact on stratum corneum. ablative fractional lasers have longer wavelengths in the range of 2,790 to 10,600 nm and lead to full-thickness destruction of skin. Whereas nonablative fractional lasers

have wavelengths ranging from 1320 to 1927 nm and leave a functionally and histologically intact stratum corneum compared to nonablative fractional lasers.

Ablative fractional lasers are usually associated with greater efficacy but longer recovery time and a higher risk of complications (Table 7).

Table (7): Fractional Lasers for acne scarring

Non-Ablative Fractional Lasers (NAFL)	Ablative Fractional lasers (AFL)
➢ Erbium-doped (1,550 nm)	➢ Erbium: YAG (2,940 nm)
➢ Erbium: Glass (1,540 nm)	➢ CO2 (10,600 nm)
➢ Erbium (1,340 nm)	➢ CO2 (10,600 nm) + Bipolar Radiofrequency
➢ Nd:YAG dual mode (1,440/1,320 nm)	➢ YSSG (2,790 nm)
➢ Alexandrite picosecond (755-nm)	
➢ Nd:YAG/KTP picosecond (1,064/532 nm)	
➢ Diode (915 nm) + Bipolar Radiofrequency (needle)	

1.2.2. Non-Ablative Fractional Lasers (NAFL):

In 2004, Fractional Photothermolysis (FP) technique was developed by Manstein and his colleagues to treat fractions of the skin without destroying neither the entire skin depth nor the whole treated surface area. Fractional lasers are used to induce zones of microscopic thermal injury that comprise marked areas of tissue denaturation 50–100 μm in diameter, which are surrounded by normal viable tissue. The adjacent viable tissue is spared to allow the rapid lateral migration of keratinocytes, leading to complete re-epithelialization of the epidermis within 24 hours. Hence, skin remodeling can be achieved with a lower risk of complications and a higher degree of efficacy. During each treatment session, about 16-32% of the skin surface is targeted; this proportion is determined by the density settings of the device and the number of passes. Usually, after fractional resurfacing, the stratum corneum remains intact, and the risk of adverse effects associated with ablative skin resurfacing, including infection and scarring, are therefore reduced. In addition, the depth of COL remodeling that is associated with the use of fractional resurfacing can be deeper than the conventional aggressive laser resurfacing procedure.(85)

Introduction

1.2.2.1. Fractional non-ablative erbium-doped fiber (1550 nm):

The 1550 nm erbium-doped fiber laser (Fraxel SR 750, Solta Medical) was the first available system that can induce arrays of columns of thermal damage, known as microscopic treatment zones (MTZs). The newer generation, called Fraxel Re: store®, can even penetrate deeper into the dermis without using the blue dye as a tracking system. The unique feature of fractional resurfacing is its ability to induce thermal damage and subsequent dermal remodeling in columns, thus leaving the adjacent tissue surrounding each MTZ intact and facilitating subsequent healing.(83, 86)

The selection of energy levels is based on the desired depth of penetration, which corresponds to the depth of the post-acne scars. Treatment densities and energy fluences can be adjusted in terms of the extent of acne scarring, its anatomical location, skin tone, and color. The energy and the MTZ density may be limited by patient discomfort, and the additional use of an air-cooling system may lead to greater patient tolerance.(87) The use of topical and parenteral anesthetics is frequently indicated because of the considerable degree of discomfort caused by high-energy settings. Also, an air-cooling device from Zimmer® is usually incorporated with fractional laser systems to cool the skin concomitantly and help the patients to tolerate higher energy levels, thus facilitating the treatment of the deeper parts of acne scars.(88)

Kim and his colleagues were the first to report the treatment of acneiform scarring in ten patients using the fractional 1,550 nm fiber-based laser system. Patients received four to five treatments administered at weekly intervals. Treatments were done with an energy setting of 8 mJ/MTZ and a final density of 2000 MTZs per cm^2. According to objective quantifiable criteria and patient feedback, they concluded that the treatment significantly improves the appearance and contour of acneiform scars.(89)

In 2006, Geronemus reported the treatment of acne scarring with the same nonablative FP device. Patients with diverse morphology of scars, ranging from ice-pick to boxcar and rolling scars, were enrolled in a series of five treatments at 1- to 3-week intervals. Mean clinical improvement ranged from 25% to 50% assessed using digital photography and from 22% to 66% assessed using high-resolution typographic imaging. No serious adverse side effects were observed.(42)

However, one year later, Alster and her colleagues reported the results for a cohort of 53 patients with mild to moderate atrophic acne scars treated with a series of FP treatments. After a series of three-monthly treatments, clinical improvement ranged from

51% to 95% in 90% of patients; side effects included transient erythema and edema in most patients, but no dyspigmentation, ulceration, or scarring was observed.(90)

A recent study reported on acne Scarring in 7 patients with Fitzpatrick skin phototypes IV-VI. A series of 4 treatments were performed using high versus low-density parameters, clinical improvement was assessed by patients and two blinded dermatologists using a global scar assessment Visual Analog Scale (VAS), which subjectively assessed how the acne scarring had changed on a 0 to 10 scale with 0 being no change and 10 representing "like normal skin.". The two blinded dermatologist photoraters observed a statistically significant improvement from both treatment densities at both week 16 and week 24 compared with baseline. Patients reported a statistically significant improvement in acne scar severity as measured by the VAS with both higher and lower density treatment settings over time. Specifically, acne scar severity was significantly improved at Weeks 8, 12, 16, and 24 compared to Week 4. The blinded dermatologists and patients did not perceive a significant difference in acne scar severity between the 2 treatment settings (higher vs lower densities).(91)

1.2.2.2. Fractional non-ablative Erbium: Glass (1,540 nm):

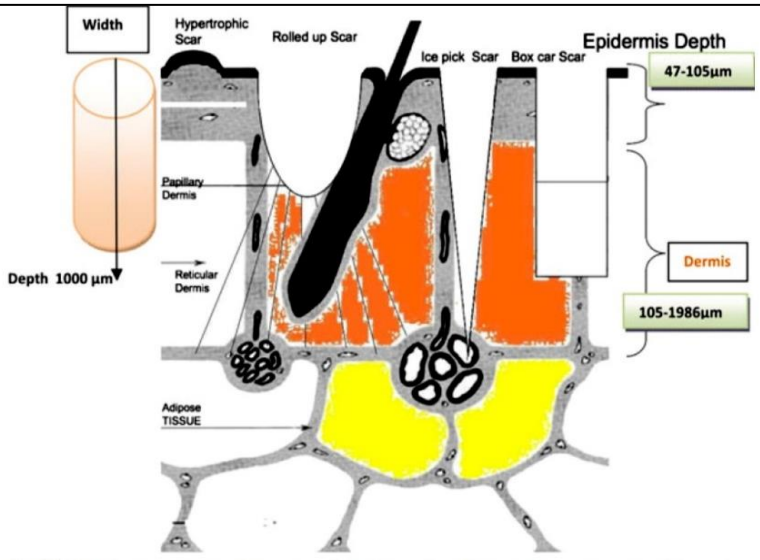
The 1,540 nm isn't that far away from 1,550 nm technology. Weiss and his colleagues were the first to report on Erbium 1540 nm laser in acne scars patients using (Lux1540, Palomar) for over 500 treatments. Three treatments at 4-week using a fluence of 50–70mJ/ microbeam with a minimum of 3 passes for each treatment site. The subjects were followed for 3 months post-treatment. Results assessed by blinded photographic evaluation showed a median of 50–75% improvement. Side effects were minimal and included mild posttreatment erythema and edema resolving within 24 hours. Pain was reported as minimal. 85% of patients rated their skin as improved.(92)

A year later, Yoo and his colleagues reported on acne scars in Asian patients. Sixteen volunteers (Fitzpatrick skin types III–IV) with mild to moderate acne scars were enrolled. Three treatment sessions using (StarluxTM 1540) were performed for each patient 4 weeks apart. A mild to moderate clinical improvement was observed in most of the patients. Moreover, the QOL of all the patients improved, and all of them were satisfied with the results of the treatment. Significant COL and elastic-fiber increases were also observed after the treatment, and side effects were limited to transient erythema

and edema, which occurred in 50% of the patients. No severe side effect was observed.(93)

Interestingly, a recent study in 2014 was performed on 35 patients to evaluate the effect of 1,540-nm fractional lasers on individual acne scars (ice-pick, boxcar, rolling) using objective assessment tools in the darkly pigmented skin. In this study, six sessions were performed with four passes per session and a dose varying from 70 to 100 mJ to maximize the Depth-to-Width Ratio (DWR) and a VAS was used to count individual atrophic scars and their final assessment was done 6 months after final laser treatment. They found that boxcar scars (52.9%) responded better than the rolling (43.1%) and ice-pick scars (25.9%), with statistically significant improvement ($p < .05$) seen in boxcar scars after four sessions. This was explained by the vertical deeper extension of Ice-pick scars into the deep dermis or subcutaneous tissue, than the depth of conventional lasers. While rolling scars, although superficial, are anchored to the subcutis by fibrosis. On the other hand, shallow (0.1–0.5 mm) or deep (≥0.5 mm) Boxcar scars, were still amenable to fractional lasers (Figure 7). However, overall VAS scores revealed significant improvement ($p < .001$).(94)

Figure (**7**): Histologic depth of erbium-doped glass laser in relation to various atrophic acne scars. Adapted from (94)



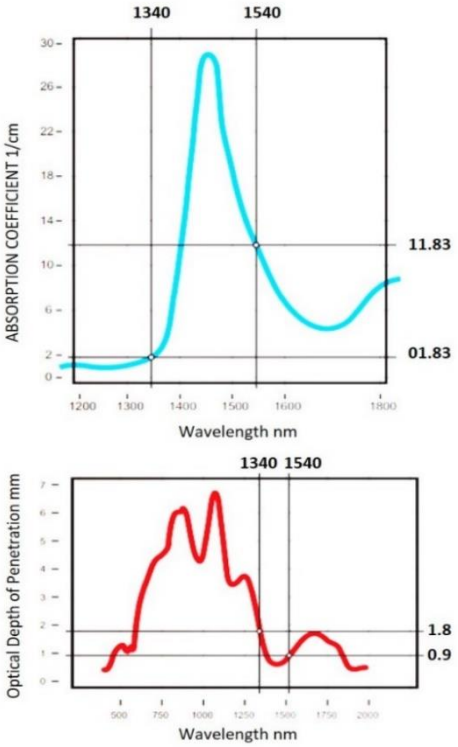
However, the density of the total MTZ may be of more importance than the energy of the fractionated laser. The use of epidermal air cooling may also decrease the incidence of post-procedural dyschromia.(95) The revolutionary advances in fractional technology have resulted in the development of new devices with different laser and Radiofrequency modalities.

Introduction

1.2.2.3. Fractional non-ablative Erbium (1,340nm):

The concept behind using the 1,340 nm wavelength came from a very old paper published in 1973. According to Hale and Querry, the coefficient of water absorption for the wavelength 1340 nm is (1.83), while 1540 nm is much higher (11.83). Thus, the 1,340 nm can penetrate deeper than 1,540 nm as shown in Figure (8 – simplified from Hale and Querry, 1973).(96)

Figure (8): simplified diagram comparing 1,340 and 1,540 nm wavelengths water absorption and depth of penetration. Adapted from (97)



In 2016, a single study by Cachafeiro and his colleagues reported on a comparison of nonablative fractional Erbium Laser 1,340 nm and Microneedling for the treatment of atrophic acne scar in a randomized clinical trial. Forty-two patients with atrophic facial acne scars were randomized to microneedling (Dr. Roller; MTO Importer and Distributor; 20 patients) or 1,340-nm nonablative fractional erbium laser (ProDeep, Etherea/Industra platform; 22 patients) treatments, receiving 3 sessions performed monthly. Two blinded dermatologists applied the validated "Quantitative Global Grading System for Post acne Scarring" scale, before, 2 months, and 6 months after the treatment. Side effects were recorded at each follow-up visit and patients' satisfaction was evaluated. Both groups showed a significant improvement, and there was no statistically significant difference between the results of both therapies. The erythema after each session was longer in the laser group PIH was only observed in 2 participants skin type IV, which were in the laser group. No patients in the microneedling group developed PIH.(97)

1.2.2.4. Fractional Nd: YAG Dual mode (1440/1320 nm):

Although this system has been introduced to the market more than 10 years ago, only two studies have demonstrated the effects of Fractional Nd: YAG Dual mode (1440/1320 nm) on acne scars.

The first study by Lloyd and Tanghetti reported on comparison of affirm 1320/1440 nm versus 1320 nm for the treatment of acne scars both clinically and histologically. Twenty-one patients with acne scars were enrolled in a multi-center study using the Affirm laser (Cynosure, Inc.) in two different treatment modalities: 1320/1440 nm (Multiplex) and the 1320 nm both equipped with a T-350 CAP array with adjunct cooling (SmartCool® Cynosure, Inc.). Treatments were given at 3-week intervals for a total of 5 treatments. The results were evaluated using photographic comparisons at baseline and 3 months to determine efficacy employing a macro-assessment grading scale, 0 (no improvement) to 4 (excellent). Also, biopsies were obtained and evaluated at 24 hours and 3 months. Amazingly, results were encouraging, and all patients completed the study and noticed improvement in their acne scars. There was greater improvement noted with the Multiplex by both physicians and patients. The histology found thermal damage in the dermis 24 hours following the multiplex treatment and new COL formation was seen at 3 months.(98)

However, the results of the second study by Babilas and his colleagues were not encouraging. They reported on their clinical experience with treatments using this dual-mode laser device (1,440/1,320 nm) in hypertrophic scars, acne scars, and facial wrinkles. Thirty-six patients suffering from hypertrophic scars ($n = 7$), acne scars ($n = 9$), and wrinkles ($n = 20$) were treated for three to eight sessions every 6 weeks. Retrospective evaluations by two blinded investigators based on follow-up photographs and patient self-assessment were graded at 3-month follow-up after the final treatment in comparison to baseline as worse (-1), equal (0), slightly improved (score 1), improved (score 2), or significantly improved (score 3). In total, nine patients were treated for acne scars; five patients (55.56%) rated the results with a score of (1), and four patients (44.44%) rated the results with (0). Physicians rated the results with 3 in two patients (22.22%), with 2 in four patients (44.44%), and with 1 in three patients (33.34%).(99)

1.2.2.5. Fractional Alexandrite picosecond (755 nm):

Recently, an innovative optical attachment for the picosecond laser, a diffractive lens array, has been developed that gauges the distribution of energy to the treatment area. This specialized optic affects more surface area, has a greater pattern density per pulse, and may improve the appearance of acne scars.

Brauer and his colleagues were the first to describe the use of a picosecond 755-nm laser with a diffractive lens array in the treatment of facial acne scarring. Fifteen

women and 5 men (mean age, 44 years; age range, 27-61 years) with Fitzpatrick skin types I through V and facial acne scarring were enrolled, however, only 17 patients completed the study. Patients received 6 treatments every 4 to 8 weeks; based on their Fitzpatrick skin type, with a 755-nm picosecond laser (Picosure™, Cynosure) with a spot size of 6 mm, fluence of 0.71 J/cm2, repetition rate of 5 Hz, and pulse width of 750 picoseconds in combination with a diffractive lens array. Patients were asked to rate their satisfaction with improvement in overall appearance and texture on a 4-point scale (0 indicating not satisfied; 1, dissatisfied; 2, satisfied; and 3, extremely satisfied) before their final treatment visit and at 1 and 3 months after the sixth treatment. Three independent masked physician evaluators used a 4-point scale (0 indicating 0%-25%; 1, 26%-50%; 2, 51%-75%; and 3, 76%-100%) to assess improvement at 1 and 3 months after 6 treatment sessions. Patients were satisfied to very satisfied with overall appearance and texture at the final treatment session and at 1 and 3 months, with scores on a range of 0 to 3 of 2.33, 2.45, and 2.2 for overall appearance and 2.33, 2.36, and 2.2 for texture. Masked physicians' assessment was performed, with mean scores of 1.5 and 1.4 at 1 and 3 months, respectively. Moreover, evaluation of histologic specimens from 2 patients at 3-month follow-up revealed notable changes from baseline, specifically, within the dermis there was an elongation and increased density of elastic fibers, an increase in COL III, and an increase in deposition of mucin throughout all the layers of the dermis.(100)

In 2016, Tanghetti reported in detail on the histology of skin treated with a Picosecond Alexandrite Laser and a fractional lens array. Immediate post-treatment histology demonstrated unique well defined intra-epidermal spaces (vacuoles) resulting from areas of Laser-Induced Optical Breakdown (**LIOB**), which is consistent with a localized plasma formation in the epidermis initiated by the melanin absorption of the high energy picosecond light. This could directly stimulate an epidermal repair mechanism that results in improvements in dyspigmentation and acne scars with new COL, elastic tissue, and mucin.(101)

Two recent studies; one of which performed in a large cohort of patients have confirmed clinical improvement similar to the first report published by Brauer and his colleagues. Unfortunately, the first study reported only 3 acne scars patients. However, Huang and his colleagues reported on a retrospective photographic review for 42 Asian patients; assessed by 2 blinded dermatologists for overall skin quality improvement on a 5-point scale. All patients experienced improvements in scar texture and overall skin

quality after 2 to 6 sessions, with scores of +1.4, 1.45, 1.7, 1.33, 2.3, and 1.66 points after 2, 3, 4, 5, 6, and >6 treatments, respectively. There were no obvious adverse effects after treatment. The post-inflammatory hyperpigmentation (PIH) risk was 4.7% (2 of 42, both spontaneously resolved).(102, 103)

Interestingly, both results were almost similar in terms of improvement and side effects.

1.2.2.6. Fractional Nd: YAG/KTP picosecond (1,064/532 nm):

Meanwhile, in 2017, Bernstein and his colleagues reported on a novel fractional treatment with a dual-wavelength 1,064 and 532nm picosecond-domain laser, delivering a 10 X 10 array of highly focused beamlets via a holographic optic beam-splitter (PicoWay Resolve®, Syneron-Candela Corporation). Twenty-seven of 31 subjects completed the study, 19 were treated using 1,064 nm and 8 were treated at 532 nm, all having four-monthly treatments. Blinded evaluation of digital images by three physician evaluators comparing pre and 3-month post-treatment images measured efficacy using a 10-point scale. Subject self-assessment of treatment effects was also recorded. Safety was measured by recording subject discomfort scores and adverse effects. Blinded reviewers correctly identified the baseline image in 61 of the 81 image sets (75%), and baseline acne scar scores were 1.8 ± 0.7 and 1.8 ± 0.5 and decreased after treatments to 1.1 ± 0.5 ($P < 0.001$) and 1.1 ± 0.0 ($P < 0.005$) for the 1,064 and 532 nm cohorts, respectively. Post-treatment erythema, mild edema, and petechiae were the only side effects noted.(104)

This was followed by 2 studies from Chung-Ang University College of Medicine, Seoul, Korea. A Case report of a 23-year-old female presented with multiple pitting acne scars on her cheeks who was successfully treated using 532-nm (PicoWay Resolve®, Syneron-Candela Corporation). After 3 sessions repeated at 1-month interval, the scars were significantly improved, and the patient was very satisfied with the cosmetic outcome. No severe adverse effects were detected except for instant erythema and procedural discomfort, which resolved within a few hours. Furthermore, a conference paper reported on the efficacy and safety of 1,064 nm picosecond laser with Micro Lens Array (**MLA**) in 14 patients with acne scars. Unlike the previous studies, patients received five to seven sessions with 1-2-week interval and a punch biopsy was performed before treatment and at follow up in 11 patients for histologic analysis. All patients experienced both subjective and objective improvement. Histologic evaluation

showed increased dermal COL with thickening of the dermis and straightening of elastic fibers in reticular dermis after treatment.(105, 106)

Generally, downtime with NAFL devices does exist, with most patients experiencing erythema and edema for 24 to 48 hours post-procedure. Other adverse events to these devices appear to be negligible, except for the potential for post-inflammatory hyperpigmentation (PIH), which is seen more commonly in darker-skinned individuals. Proper pre-treatment and post-treatment care should minimize the risk of PIH in susceptible individuals. Transient tiny crust formation and apparent bronzing secondary to transepidermal extrusion of concentrated melanin usually last for 1-2 weeks. The incidence of other serious adverse events, including scars, is not yet reported with the NAFL devices.(107)

1.2.3. Ablative Fractional LASERs (AFL):

Ablative fractional Laser (AFL) resurfacing is a new technique that bridges the gap between NAFL and ablative laser dermabrasion.(108, 109) The definition of AFL is based on the level of damage. AFL destroys the whole column of skin including epidermis and dermis while NAFL only coagulates the dermis and keeps the epidermal intact. There was a significant reduction in volume and scar depth according to image analysis after carbon dioxide (CO_2) AFL.(110) AFL provides a more aggressive FP treatment option for difficult cases and is useful for severe atrophic acne scarring.

Although, the 1,550-nm erbium fiber laser provided a safe and easy procedure in treating atrophic facial acne scars in type III and IV skin.(111) However, it was found that even multiple sessions of NAFL cannot exert a significant tightening effect in cases with deep atrophic scars and skin laxity. Thus, the popularity of nonablative fractional 1,550-nm erbium fiber laser declined after the introduction of AFL, including 2,940-nm Er:YAG and CO_2 lasers for severe scarring.(112)

1.2.3.1. Fractional Erbium: Yttrium-Aluminum-Garnet (2,940-nm):

The wavelength of the Er: YAG laser (2,940 nm) more closely approximates the absorption peak of water (3,000 nm) in the target chromophore than the wavelength of the CO_2 laser (10,600 nm). Thus, it is highly absorbed by water, so it is almost totally absorbed by a very thin, superficial layer of skin and can be used for precise and

superficial tissue ablation.(113) Although Er:YAG laser has the advantages of quicker healing, lower degree of erythema and less postoperative morbidity, the long-term results in facial rejuvenation have been disappointing. These effects are believed to be due to the much smaller band of Residual Thermal Damage (RTD) left in the dermal tissue following Er: YAG ablation compared with the deeper band of RTD created by the CO_2 laser.(114) A variety of newer laser systems have attempted to combine the precise ablation of a short-pulsed Er: YAG laser with some of the controlled thermal effects seen with CO_2 laser.(115)

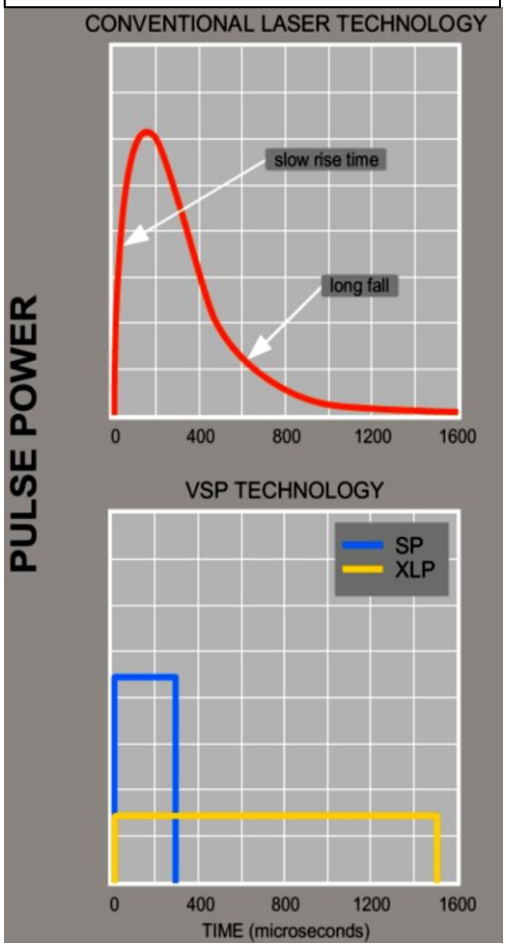
Thus, dual-mode Er: YAG laser systems with both short-pulse, ablative and long-pulse, sub-ablative coagulative components have been developed. According to the histologic findings, thermal damage extended approximately 40–50 μm beneath the vaporized layer immediately following laser treatment. This was less deep than with ultra-pulse CO_2 laser resurfacing but deeper than with conventional, short-pulsed Er: YAG laser.(116)

Clinically, the earliest study for patients treated with the dual-mode Er: YAG laser showed significant improvements in their pitted, facial acne scars, with an overall improvement of 75%. Although this study was not a side-by-side comparison, these improvements were better than those previously reported for conventional, short-pulsed Er:YAG laser resurfacing.(117) In addition, their results were similar to those reported when a CO_2 laser is used alone or combined with an Er:YAG laser.(34, 118) The ability of the dual-mode Er:YAG laser to abrade and its deep photothermal effect might have contributed to this favourable result. However, complications such as post-inflammatory hyperpigmentation, prolonged erythema, hypopigmentation, and acne flare-up, were observed after the dual-mode Er: YAG laser treatment. Post-inflammatory hyperpigmentation faded or disappeared within three months after laser treatment.(119)

In 2004, Woo and his colleagues reported on evaluating the clinical effects of resurfacing with the short-pulsed Er: YAG laser, the Variable Square Pulse (VSP) Er:YAG laser, and the dual-mode Er:YAG laser for each type of facial acne scars. A large cohort of 150 patients with different types of facial atrophic acne scars was enrolled in their study. They concluded that shallow boxcar and ice-pick scars can be treated successfully using any type of Er: YAG laser. While, in cases of rolling and deep boxcar scars, however, Er:YAG laser with a long-pulse duration for a thermal effect is needed for successful treatment.(120)

Introduction

Figure (9): Comparison of conventional laser technology platform (slow rises and long falls) and square-shaped pulse technology with variable pulse width from (121)



Later, Wanitphakdeedecha and his colleagues reported on determining the efficacy and side effects of (VSP) Er: YAG laser resurfacing for the treatment of punched-out atrophic and rolling acne scars. Twenty-four subjects with acne scars were divided into two groups and treated with two different pulse widths: 300 ms (short pulse, **SP**) and 1,500 ms (extralong pulse, **XLP**) as shown in Figure (9). Their conclusion was that a low-fluence VSP Er: YAG laser resurfacing is a promising treatment option for acne scars, with minimal risk of side effects, there were two important findings. First, the difference in clinical improvement between the **SP** and **XLP** groups; only skin smoothness improved significantly after treatment in the SP group, whereas skin smoothness and volume of acne scars improved significantly in the XLP group. Second, the discrepancy between physician evaluation and patient self-evaluation of perceived clinical improvement; physician evaluators rated a greater number of patients as achieving a higher category of improvement than the patients themselves reflecting the difference in treatment outcome expectations.(121)

A recent study reported on randomized split-face clinical and histopathological comparison of fractional ablative (2940nm) vs. non-ablative (1540nm) methods after 3 months. Eighteen patients completed the treatment with three sessions of 1540 nm fractional Erbium laser (NAFL) on one side of the face, and one session of fractional 2940 nm (AFL) on the other side. Biopsies were performed before and 3 months after treatment. Clinical, histological and morphometric evaluations were carried out. All patients presented clinical improvement with no statistically significant difference ($p > 0.05$) between the treated sides. Histopathology revealed a new organization of COL and elastic fibers, accompanied by edema, which was more evident with the 2940 nm laser. This finding was confirmed by morphometry, which showed a decrease in COL density for both treatments, with a statistical significance for the 2940nm laser ($p > 0.001$).

According to this study, three 1540 nm sessions were clinically equivalent to one 2940 nm session. The edema probably contributed to the positive results after three months, together with the new COL and elastic fibers organization. The greater edema after the 2940 nm session indicates that dermal remodelling takes a longer time than with 1540nm. It is possible that this histological superiority relates to a more prolonged effect, but a cohort longer than three months is needed to confirm that supposition.(122)

The latest but not the least was a retrospective study reporting treatment of facial acne scars with VSP Er:YAG Laser in skin types IV and V. A cohort of 80 patients, who had undergone a minimum of four sessions, were enrolled using **SP** and **XLP** modes. Patient's satisfaction with the treatment and observer's assessment of improvement (based on photographs) was graded as poor (<25% improvement), fair (25‑50% improvement), good (51‑75% improvement), and excellent (>75% improvement). Ninety-seven percent of the subjects in this study perceived fair improvement at least.(123)

1.2.3.2. Fractional Carbon Dioxide 10,600 nm:

CO_2 lasers emit light at 10,600 nm in the far-infrared electromagnetic spectrum. The emitted energy is preferentially absorbed by intracellular water, which leads to rapid heating and vaporization of tissue. CO_2 lasers were introduced in the 1960s and were initially used in the continuous wave (CW) mode for cutting tissue.(124)

Even before the fractional scanners, the development of high-energy pulsed CO_2 lasers that minimize thermal injury to uninvolved adjacent structures has revolutionized the ongoing quest for recontouring atrophic facial scars. In 1999, the first prospective analysis of clinical and histologic effects of cutaneous laser scar revision has been conducted by Walia and Alster. Their histopathology findings paralleled the clinical improvement seen. The prolonged and enhanced clinical improvement correlated directly with the degree of new COL deposition and dermal remodelling observed histologically in their study up to and beyond 12 months.(125)

In 2007, Hantash and colleagues first described the use of an ‘‘ablative’’ CO_2 fractional resurfacing device (Reliant Technologies, Inc., Mountain View, CA), which produces an array of microthermal zones (MTZs) of a customizable density and depth with a confluent pattern of ablation and coagulation extending from the stratum corneum through the dermis. In the initial in vivo study demonstrating the histologic and clinical

effects of this device, they confirmed with immunohistochemistry that persistent COL remodelling occurred for at least 3 months after treatment.(109)

In 2009, Gotkin and his colleagues reported on skin resurfacing using a new novel microablative CO_2 laser device (SmartXide DOT, Deka M.E.L.A., Florence, Italy). Although a single treatment was performed using a relatively high fluence, all patients demonstrated significant clinical improvement in wrinkles, scars, striae, lentigines and solar elastosis. However, less dramatic results were noted in acne scars and striae. Such a note was reported by Chapas and his colleagues who demonstrated much more significant clinical improvement in the overall scar depth and appearance in acne scars patients who went on to have additional treatments.(44, 126)

The previous study was followed by another report from Prignano and her colleagues using the same device. The aim of their work was to compare how different CO_2 laser fluences, by modulating the secretory pathway of cytokines, can influence the wound-healing process, and how these fluences are associated with different clinical results. The immunohistochemical assessment was performed at defined endpoints in order to obtain information about specific cytokines of the microenvironment before and after treatment. They proved that the secretory pathway of cytokines changed depending on the reepithelization and the different laser fluences (Table 8). Different but significant improvements were definitely obtained when using 2.07, 2.77, and 4.15 J/cm2, indicating fractional CO_2 laser as a valuable tool in photorejuvenation with good clinical results, rapid downtime, and an excellent safety profile.(127)

Table (**8**)**:** Results of immunolabeling scores at time 0, immediately after treatment, 3 days after treatment, and 30 days after treatment. Adapted from (127)

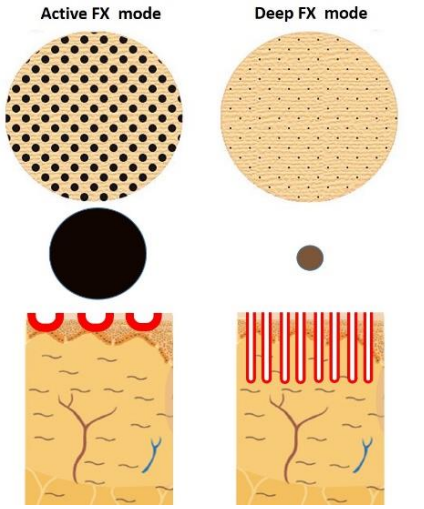
		Immediately after			3 days after			30 days after		
	Time 0	2.07 J/cm²	2.77 J/cm²	4.15 J/cm²	2.07 J/cm²	2.77 J/cm²	4.15 J/cm²	2.07 J/cm²	2.77 J/cm²	4.15 J/cm²
EGF	1	3	2	0	3	1	0	1	3	0
bFGF	0	1	0	1	1	2	1	1	3	0
PDGF	1	2	1	1	2	2	2	1	2	0
VEGF	0	1	1	0	1	1	0	1	1	0
TGF-β	0	1	0	0	2	3	0	1	1	0
Vimentin	1	3	0	0	3	2	0	1	1	0

EGF, bFGF, PDGF, VEGF, TGF-β, and vimentin immunoreactive cells were observed under light microscopy. A range from 0 to 3 was used for the score. The absence of immune reaction was scored as "0," a scarce immunoreactivity was scored as "1," an intermediate immunoreactivity was scored as "2," and an intense immunoreactivity was scored as "3."

bFGF, basic fibroblast growth factor; EGF, endothelial growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

However, the same research group came with another study; in contrast with Gotkin and colleagues, to demonstrate on both clinical and histological data obtained from their patients that there is no advantage upon treatment with 4.15 J /cm^2 compared to those obtained with 2.07 and 2.77 J /cm^2.(128)

Figure (10): Diagram illustrating Active FX & Deep FX modes



In a different study, Cho and colleagues evaluated the efficacy and safety of the combined use of two treatment modes of another fractional ablative carbon dioxide laser (Ultrapulse Encore, Lumenis Inc., Santa Clara, CA) on acne scars. Twenty Korean patients with atrophic acne scars were treated with a single session. The laser fluences were delivered to the scars using the Deep FX mode and an additional treatment using the Active FX mode was performed throughout the entire face as shown in Figure (10). The results were highly suggestive for using a combination of two different treatment modes to provide a new treatment algorithm for acne scars.(129)

In another study, the same research group reported on a randomized split-face study comparing non-ablative 1550 nm (NAFL) Er: glass and fractional ablative 10,600 nm (AFL) carbon dioxide for acne scars. A single session for only eight patients was done. Half of each subject's face was treated with Fraxel SR1500 (Reliant Technologies, Inc., Mountain View, CA) and the other half was treated with Ultrapulse Encore (Lumenis Inc., Santa Clara, CA) dual mode using the Deep FX mode on acne scars and the Active FX mode throughout the entire face. It appears that a single treatment with the CO_2 AFL is more effective than the NAFL, although the results did not reach statistical significance because of the small study sample consisting only of male patients.(130)

1.2.3.3. Fractional CO_2 (10,600 nm) + Bipolar Radiofrequency (RF):

Similar to innovations in Er: YAG laser system, there were many on fractional CO2 laser devices, aimed to maximizing the ablation effects while controlling the residual thermal effects.

A new fractional CO_2 laser (SmartXide^2, DEKA M.E.L.A., Calenzano, Italy) combines a scanning system to produce thermal effects in micro areas with the emission of a bipolar radiofrequency. The system generates perfectly controlled energy pulses (DOTs) by managing the "energy per pulse" parameter, the "DOT spacing" between two microscopic wounds, and the pulse duration (known as "dwell time").

Introduction

Additionally, bipolar RF (through managing "power" and "time" parameters) have been added simultaneously to the fractional CO_2 laser emission to achieve greater therapeutic efficacy thanks to the synergy of the two methods (Figure 11). (131)

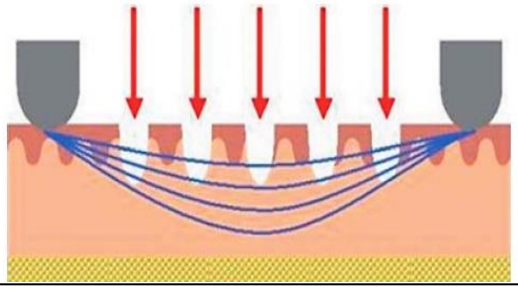


Figure (**11**): CO_2 & RF Bioplar Synergy. Adapted from (131).

This technology also offers different pulse shapes (such as the **S**-pulse, **D**-pulse, and **H**-pulse) as shown in Figure (**12**); which play an important role in ensuring both superficial ablation of the epidermis and the release of heat deeper down in the dermis. The "Smart Stack Mode" is an additional parameter which repeats the laser pulse in the same DOT for a maximum of five times before moving on to the next one, to increase the thermal effect.

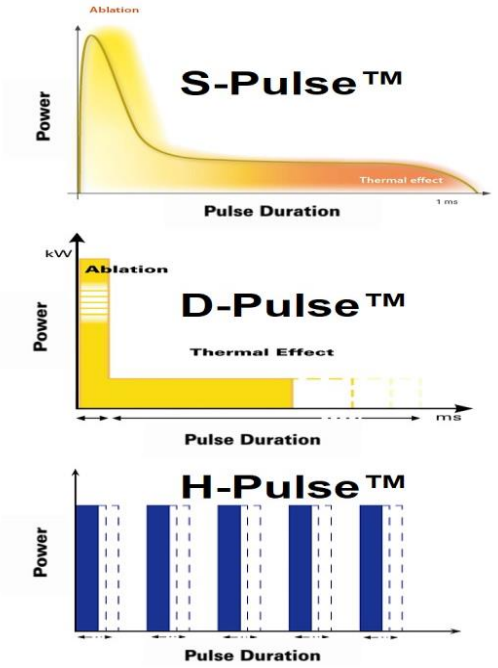


Figure (**12**): S-Pulse, D-Pulse and H-Pulse modes illustration.

Tenna and her colleagues reported on this innovation of combined use of fractional CO_2 laser and radiofrequency waves to treat acne scars in a pilot study on 15 patients. Patients underwent two treatment sessions 4 weeks apart, with a dwell time of 1 ms, a DOT spacing of 500 mm, Smart Stack 2/3 and an energy per DOT of almost 45 mJ. Radiofrequency outputs were 20‑30 Watts and duration 2‑3 seconds. D-pulse was preferred for a deeper action in acne scars patients. Photographs were taken and objective examination, performed at the 3-month and 12-month follow-ups, was carried out by comparing the results with the score of the GAIS (Global Aesthetic Improvement Scale) used as a reference parameter. The GAIS evaluation was 20% "Very Much Improved", 53.3% "Much Improved" and 26.6% "Improved" at the three-month follow-up, while at the 12-month follow-up 13.3% "Very Much Improved", 60% "Much Improved" and 26.6% "Improved", without a statistically significant difference in

the GAIS scores between 3-month and 12-month (p= 0.093). No post-inflammatory hyperpigmentation was observed.(131)

Later, another study reported using the same combination including D-Pulse and RF in a case series. Only five patients with acne scars were enrolled for three treatment sessions with an interval time of 2 months. All patients were photographed and assessed by an independent physician on a five-point scale for improvement in comparison with baseline from 0 to 4, considering 0 as no improvement versus baseline, 1 as a moderate improvement, 2 as a good improvement, 3 as a very good improvement, and 4 as an excellent improvement. One patient was recorded as 4, 3 patients were 3 and one patient as 2. 80% of patients reported a mild burning sensation during treatment but immediate good clinical appearance.(132)

1.2.3.4. Fractional Yttrium Scandium Gallium Garnet (2,790-nm):

The 2,790 nm yttrium-scandium gallium-garnet (YSGG) laser is an ablative fractional laser that targets water molecules in the superficial layers of the skin. Its wavelength produces an intermediate degree of thermal energy relative to the 10,600 nm CO_2 and 2,940 nm Er: YAG lasers. It has a water absorption coefficient roughly 5 times that of CO_2 and third that of Er: YAG as shown in Figure (13). Therefore, it offers a moderate balance of ablation, coagulation, and side effects.(133)

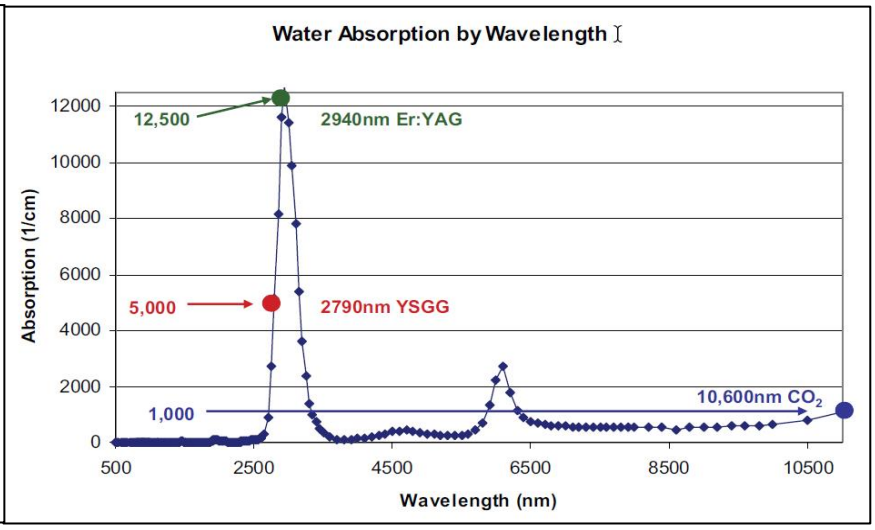


Figure (13): Graphic showing range of wavelength specific absorption coefficients of water. Adapted from Ross et al (133)

The 2,790 nm YSGG laser device also has both ablative (confluent) and fractionally ablative capabilities which can be combined in the same treatment session. Combination treatment results in vaporization of the entire superficial epidermis, coagulation of the deeper epidermis, and fractional coagulation in the deeper dermis. It

induces a reproducible layer of 40 mm of coagulation at both sides of the ablative microcolumn. The ablative microcolumn is consistently 300 μm in diameter and can be as deep as 1 mm or more of penetration depending on the energy (mJ/cm2) delivered to the tissue. Therefore, it offers a moderate balance of ablation, coagulation, and side effects. The comparison of the photothermal effects of the combination confluent and fractional-ablative 2,790 nm YSGG laser, fractional CO_2 and Er:YAG lasers are shown in Figure (14).(134)

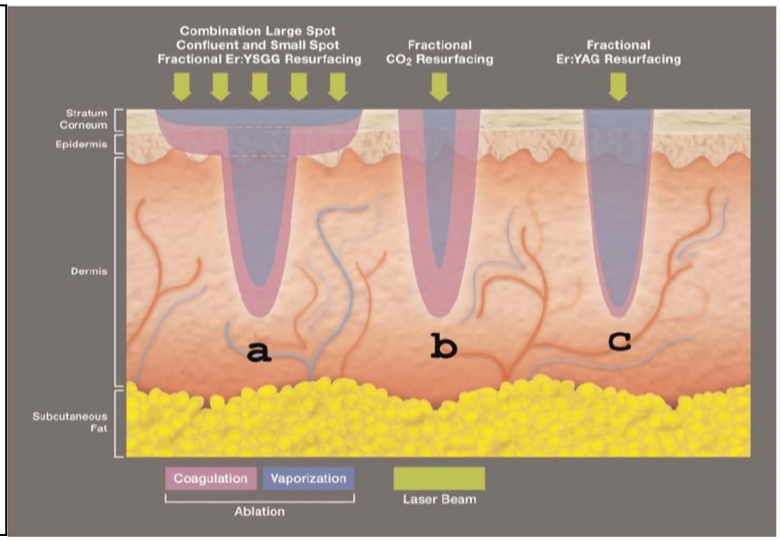


Figure (14): **(a)** Combination large spot confluent and small spot fractional Er:YSGG resurfacing and comparison with **(b)** fractional CO_2 resurfacing and **(c)** fractional Er:YAG resurfacing. Adapted from (134)

Kim was the first to report treatment of acne scars in Asian patients using a 2,790-nm. Twenty participants with skin phototype IV and atrophic acne scars were treated with two sessions of 2,790-nm (Pearl Fractional™, Cutera Inc., Brisbane, CA) ablative fractional resurfacing laser at a 6-week interval. Treatments consisted of a single pass with a focused tip and a single pass with a defocused tip. An additional pass using defocused beam mode was performed over scars. Objective and subjective (clinical evaluation by two blinded dermatologists) assessments were obtained at baseline and 1 and 3 months after the final treatment. At the 3-month follow-up, 70% of the participants were rated as having at least 50% to 89% improvement of scars. Mild erythema was the most common adverse effect, observed in 30% of participants, but resolved completely in an average of 5 days.(135)

In a different study, Kimura and his colleagues have been concerned with the biophysical effects of fractional resurfacing with YSGG Laser in Japanese patients with acne scars. Five Japanese patients underwent one session, using only a single pass per treatment site. All patients recorded a feeling of skin tightness in treated areas. There was a clinically noticeable improvement in the macroscopic appearance of acne scars at 4

weeks post-treatment and the number of ice pick scars was significantly reduced in all patients.(136) However, there was no statistical details reported on acne scars improvement, most probably due to the very small size cohort and the focus on biophysical evaluation.

1.2.4. Fractional Diode (915 nm) + Bipolar Radiofrequency:

The fractional 915-nm diode laser and bipolar RF coupled with surface cooling lead to coagulation at a depth of about 1–2 mm in the deep dermis. It was postulated that the fractional laser preheats the target area such that the subsequent RF energy is drawn towards the heated target deep in the dermis while the superficial part is protected by contact cooling. The use of both devices in combination aims to enhance COL synthesis in the scar indentation at deeper layers by infrared laser and some degree of surface ablation of the scar edges and COL remodeling by fractional RF. The enhanced COL synthesis at deeper layers may improve the appearance of scars by elevating the deeper part of the scars, resulting in a more uniform skin surface and improved texture, pore size, and pigmentation irregularity. This treatment - Sublative Rejuvenation (SR) - provides a coagulative (nonablative) effect limited to the mid-dermis in addition to an ablative injury to less than five percent of the epidermis (Figure 15).(137)

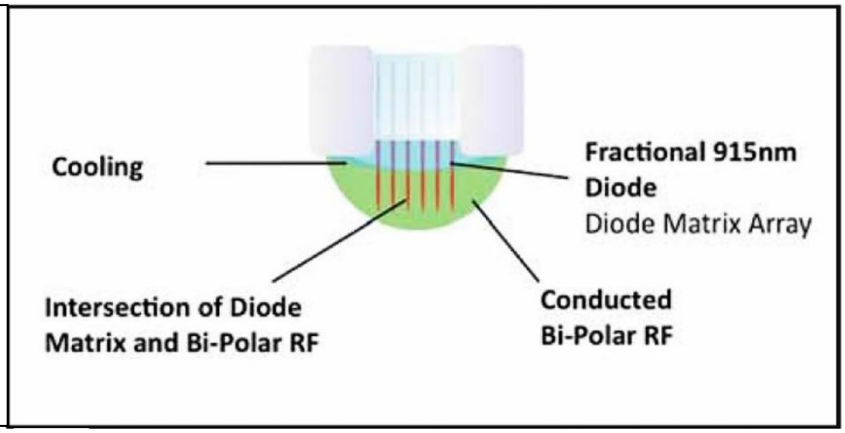


Figure (15): Schematic of Combination Bipolar Matrix Array and Bi-Polar RF (DLRF). The intersection of the two modalities creates thermal bands at 1.5mm into the dermis causing COL contraction. Figure courtesy of Syneron Medical LTD. Adapted from (137).

In 2011, Taub and his colleagues reported on this technology in 20 patients with acne scars. Patients received up to five treatments with Sublative fractional bipolar RF and bipolar RF combined with diode laser using (Matrix IR, Syneron Medical Ltd., Yokneam, Israel). Efficacy and adverse effects were assessed by physical examination at each treatment visit and at the 4- and 12-week follow-up visits. The evaluation was one month after three treatments and immediately before the 4th treatment four. Acne scars significantly improved one month after three treatments and improvement persisted for at least 12 weeks after the fifth treatment.

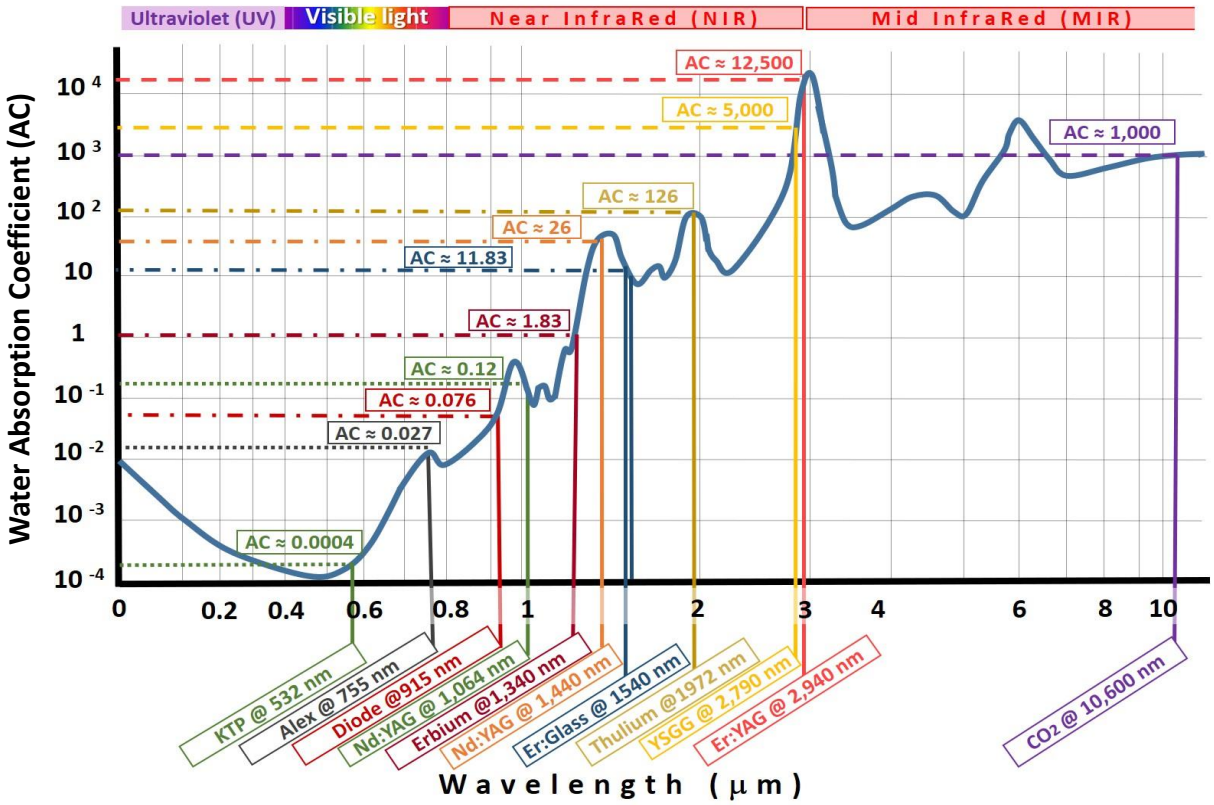
Introduction

Improvement was not affected by skin type. Adverse effects were limited to transient erythema and edema.(137)

In another cohort of 24 Asian patients with acne scars, Yeung and his colleagues conducted a similar study. Twenty patients completed the study. Modest but statistically significant improvement was noted in acne scars, with the mean grade decreased by 29% ($P < 0.001$), and 52% were rated with at least moderate objective global improvement at 3 months. Mean pain score was 2.6 on a scale of 0–4. PIH occurred mainly over bony areas in 6.5% of all treatments. Subjective improvement was moderate to significant for 36.8% of patients, and 63% reported being satisfied with the treatment results at 3 months despite considerable pain level.(138)

Lasers differ in terms of wavelengths and water absorption coefficients (Figure 16) which influence the histologic depth extents with respect to spot size, pulse duration, fluence, density, and cooling mechanisms.

Figure (16): Water Absorption Coefficients among common laser Wavelengths



Despite the many fractional laser studies in acne scars, some basic tenets in the treatment of acne scarring must be emphasized. The first is that there is no “magic wand” therapy for all cases; each scar and each patient must be treated individually, and scar topography is the target of most interventions. The second tenet is that deep scars may invariably require an excisional biopsy, but even this leads to a modified contour, and the scar is not completely effaced.(94)

This should restrict the enthusiasm for using fractional laser technology, which is one of the treatment options, as a single modality treatment.

In our opinion, optimizing the depth width Ratio while using the fractional ablative and non-ablative Lasers should be the new focus of research in correlation to the acne scar type, i.e., the laser parameters should be changed according to the type and depth of treated scars rather than using fixed parameters. Theoretically, setting Laser parameters in a protocol that allows targeting as deep as safe in the scarring areas then going superficial in the consecutive sessions, while using other parameters that help regeneration in the vicinity of the scars, would be valuable.

1. JUSTIFICATION

Justification

2. Justification:

The carbon dioxide and erbium lasers have been the gold and silver standards for acne scars treatment. The idea of combining both carbon dioxide and erbium lasers appeared even before the era of fractional lasers. McDaniel et al combined both non-fractional lasers for resurfacing of perioral rhytids comparing it to using carbon dioxide lasers alone and concluded that carbon dioxide laser resurfacing followed by 3 passes of erbium laser reduces the duration of crusting, swelling and itching when compared to carbon dioxide laser resurfacing alone with no significant difference in outcome.(139)

As with selective Photothermolysis, a major advance in the field came with incorporation of Micro Thermal Zones (MTZs) grids that spare islands of skin with an attractive treatment efficacy to downtime healing (5-7 days) ratio. Application of these fractionated resurfacing to carbon dioxide and erbium lasers allowed deeper penetration into the skin.(85)

Later in 2010, Cho et al tried out the combination of fractional lasers, carbon dioxide, and erbium-doped laser, for treating mild acne scars. They reported longer post-laser erythema and hyperpigmentation, without precise pathogenesis. However, they suggested that these unexpected outcomes may have resulted from bulk heat damage to the surrounding tissues by heat stacking and recommended further studies to determine the optimal treatment parameters and reduce unexpected adverse reactions.(140)

Platelet-rich plasma (PRP) is a high concentration of platelets in a small volume of plasma. PRP contains various growth factors and cytokines released by platelets, and those substances play a critical role in all aspects of the wound healing process. Among the stored mitogenic factors essential for wound repair are PDGF, TGF-β, VEGF, bFGF, EGF, and IGF-1. These are variously involved in stimulating chemotaxis, cell proliferation, and maturation. All of them are potent angiogenic factors and endothelial cell mitogens. PRP has been widely used in many areas of medicine, including orthopedics, sports medicine, dentistry, otorhinolaryngology, neurosurgery, ophthalmology, urology, wound healing, and cosmetic and maxillofacial surgery.(141)

Few studies have discussed the combination of topical or intradermal injections of PRP as an adjunct treatment after a single modality of fractional laser surfacing in post-acne scars given its potential to improve repair and regeneration.(57-59, 142-144)

Up to date, there are no studies on a combination of AFL and NAFL followed by PRP in the same session.

2. HYPOTHESIS

3. Hypothesis:

We hypothesize that a combination of LASERs (AFL; Fractional CO2 and NAFL Fractional Er: Glass) would better improve a wider variety of post-acne scar types, depth, and width, if applied simultaneously followed by PRP. Such a combination would maximize improvement and minimize the previously reported adverse reactions for LASERs combination, especially if confining the AFL pulses and PRP injections only to the atrophic post-acne scars while applying NAFL for the entire face. Such a hypothesis would be described as follows:

a. On clinical basis, Patients' self-assessment questionnaire would show a significant difference when comparing their before and after 1-month SCARS and FASQoL scores as well as a significant difference in Blind assessors Acne scars severity between before and 3-month after 4^{th} sessions photographs.
b. On histopathological basis, higher immature COL III and lower mature COL I would be detected in specimens treated with combined LASERs and PRP when compared to combined LASERs and NSS or control specimens as well as a similar significant change in MMP-2.

3. Objectives

4. Objectives:

4.1. Primary Outcome Evaluation:

4.1.1.Change in Patients' response denoting early Clinical improvement

Calculation of the change in two tools (Table 9 & 10); Self-assessment of Clinical Acne-Related Scars (SCARS) tool which is composed of 2 Visual Analog Scales (VAS) and 5 questions for assessing Acne Scar Appearance and Severity through two parts; Part.1: a) Active Acne VAS and b) Atrophic Acne Scar VAS, Part. 2 Assessment of Acne Scar Appearance and Severity (5 questions), and Facial Acne Scar Quality of Life (FASQoL) tool (10 questions). Patients will record their responses before the 1st session and on the date of 2nd session.(29)

4.2. Secondary Outcomes Evaluation:

4.2.1. Final Clinical improvement

The change in scores comparing 2 sets of 3 digital photographs for each patient evaluated by 4 blind assessors on a (0-10) scale, before 1st session and 3-month after 4th session. The same Atrophic Acne Scar VAS will be used by patients for self-assessment 3-month after 4th session and compared to their Atrophic Acne Scar VAS before 1st session.

4.2.2. PicroSirius Red Stain (PSR) under circularly polarized microscopy

Quantitative evaluation of COL III and COL I in biopsies from scars by circularly polarized microscopy after staining by PicroSirius Red (PSR) Stain will be performed. This will be numerically evaluated using the ImageJ computer program on digital pictures.

4.2.3. Immunohistochemical evaluation of COL I, COL III, and MMP-2

Evaluation by a blinded histopathologist using a 5-grade scale (0, +/-, +, ++, +++) for all Immunostained slides will be performed and arranged for comparison among control, LASER with NSS and, LASER with PRP specimens.

4. Materials and Methods

5.1. STUDY DESIGN

5.1.1. Type and Design of the study

Title of the study: "Combined Fractional LASERs Resurfacing with Platelets Rich Plasma (PRP) for treating Post-acne atrophic scarring".

Promoter of the study: Dr. Ahmed Abdelsalam Youssef Ibrahim. LASER Unit, Cosmetic Surgery Clinic (CSC), Kuwait

Participating center: LASER Unit, Cosmetic Surgery Clinic (CSC), Kuwait

Principal Investigator: Dr. Ahmed Abdelsalam Youssef Ibrahim

Research collaborators: Prof. Dr. Alberto Calligaro (Italy), Prof. Dr. Paolo Bonan (Italy).

Coordinating researcher: Prof. Dr. Lluis Puig, Director of the Department of Dermatology of HSCSP.

Ethical Committee for Clinical Research: Research Ethical Committee of the CSC.

ClinicalTrials.gov ID: NCT03809416

Study illness: Adult Atrophic Post-acne scars

Duration of the treatment: 12 weeks of treatment, with 12 week-follow-up

Frequency of clinical visits: basal visit, week 4, 8, 12 (treatment) and week 24 (follow-up without treatment)

Sample size: 32 patients diagnosed with Atrophic Post-acne scars

Period of inclusion: October 2017 - June 2019

Prospective, interventional, observer-blinded, intra-patient study, single center.

All the participants had 3 excision biopsies from the three most marked scars immediately after the first treatment. One biopsy of an area neither LASERs nor PRP where used served as a control. The second biopsy site was treated only by LASERs combination, while the third biopsy was treated by both LASERs combination and PRP.

The protocol was designed as an observer-blinded, intra-patient study. The four clinical blind assessors did not know which set of photographs were taken before or after treatments. The histopathologist (investigator) blindly evaluated the 3 biopsies from each patient.

Patients continued to receive another 3 similar treatment sessions of combined LASERs and PRP, without biopsies, for 12 weeks (from the baseline visit to the 12th week) and were then followed up for another 12 weeks (week 24).

The trial for each patient ended 24 weeks after the initiation. Patients kept on visiting the Dermatology Department – LASER Unit of CSC to continue their evaluation.

5.1.2. Flow chart of the study

The flow chart of the study is described in Figure (17).

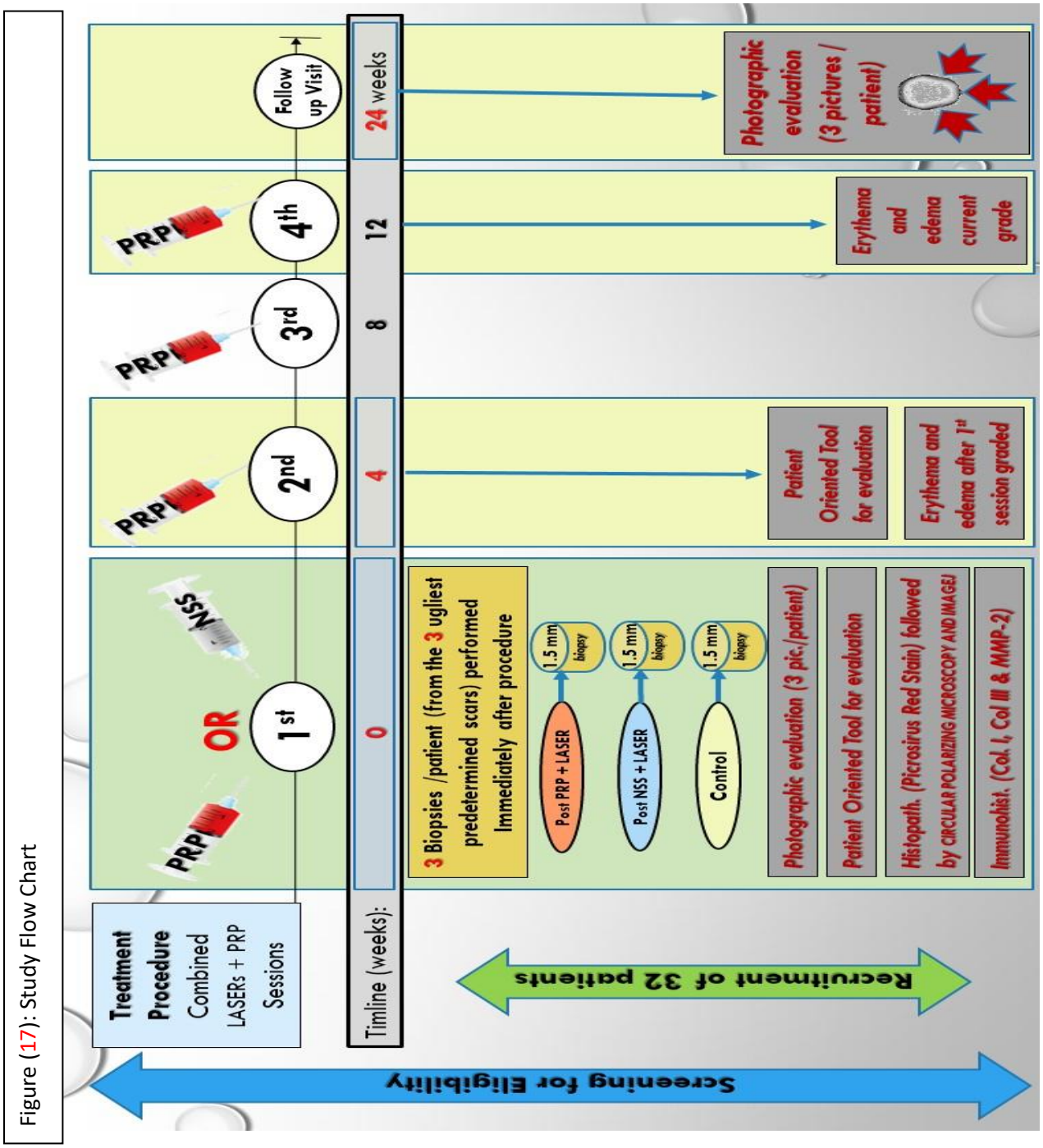


Figure (17): Study Flow Chart

5.2. Financing of the study

Only the Immunohistochemical kits were funded by DEKA M.E.L.A. S.r.l. in Florence. The rest of the work was funded by the principal investigator.

5.3. Patients

Thirty-two patients (mean age 31.6, range 19–46; Fitzpatrick skin type III, IV and V) with atrophic post-acne facial scars were enrolled in this study from our Cosmetic Surgery Clinic, Kuwait during the winter season.

5.3.1. Inclusion Criteria:

1. Patients 18 years or older with atrophic post-acne scars.
2. Patients without surgical &/or LASER resurfacing treatment for post-acne scars within the last 6 months.
3. Provision of signed informed consent.

5.3.2. Exclusion Criteria:

1. Pregnancy

2. Present or past history of hypertrophic scars or keloids

3. Present or past history of photosensitivity dermatoses including Connective Tissue Diseases.

4. Present herpes infection

5. Present history of anemia (HGB < 10 g/dl), thrombocytopenia &/or platelets dysfunction.

6. Patients receiving isotretinoin within the last 3 months, NSAIDs within 72 hours of the procedure, anticoagulants &/or systemic use of corticosteroids within 2 weeks.

5.3.3. Sample Size Calculation

For this study, 32 patients were enrolled. Minimal sample size calculation was based on Objectives a.1 and b.1, to perform the comparison of two means in a matched paired data design, with a difference of one point between both means, standard deviation of 1. Type I error was stipulated at 5% (alpha = 0.05), bilateral comparison was pre-determined, and Type II error was stipulated at 10% (beta = 0.90). We assumed a balanced design and a percentage of losses not exceeding 30%.

5.3.4. Recruitment of Patients

A social media campaign to raise awareness of the value of LASERs combinations with PRP was launched to facilitate recruitment for candidate participants. More than 100 patients were responding to the campaign messages, however, on applying inclusion and exclusion criteria, only 32 patients met the criteria to be enrolled.

5.3.5. Identification of the patients

Patients were identified by an exclusive anonymized file identification code i.e. patient's file number in the center, and another study code in chronological selection order.

5.3.6. Criteria for withdrawal and Replacement

5.3.6.1. Withdrawal Criteria

Follow-up of all patients included up to 24 weeks whenever possible. The reasons for premature withdrawal of a patient from the study could be the following:

- Decision of the patient to withdraw due to:
    a. Intolerable pain on procedure.
    b. No significant improvement.
    c. Postprocedural side effects e.g., erythema exceeding expectations and explanations by the investigator.
    d. Inability to comply with the study timeline.
- Decision of the researcher for the benefit of the patient, for medical reasons (related or not related to the treatment) or because of insufficient response. Especially if an adverse effect e.g., acne eruption, which required the prescription of a medication incompatible with the objective of the study.

5.3.6.2. Replacement of Patients

Patients withdrawn prematurely or lost for follow-up were not to be replaced.

5.4. Concomitant Treatments

Any concomitant treatment prior to or existing in the baseline visit, any new treatment or changes during the study was noted.

5.4.1. Prohibited treatments

The following medications were prohibited within 14 days prior to enrolment or at any time during the 12-week treatment period:

• Photosensitizing Medications:

1. Coal Tar and Derivatives
2. Contraceptives, Oral and Estrogens
3. Non-Steroidal Anti-Inflammatory Drugs
4. Phenothiazines
5. Psoralens
6. Sulfonamides
7. Sulfonylureas
8. Thiazide Diuretics
9. Tetracyclines
10. Tricyclic Antidepressants

• Anticoagulant Medications

1. Apixaban (Eliquis)
2. Dabigatran (Pradaxa)
3. Edoxaban (Savaysa)
4. Fondaparinux (Arixtra)
5. Heparin (Fragmin, Innohep, and Lovenox)
6. Rivaroxaban (Xarelto)
7. Warfarin (Coumadin, Jantoven)

• Corticosteroids by any route (topical, oral, IM or IV)

If it was considered medically necessary to administer any prohibited medication during the 3-month treatment period, the patient was excluded from the study; however, the patient was able to continue treatments later.

5.4.2. Authorized treatments

All medicines other than the prohibited medications indicated in the previous section were authorized. Topical treatments to be used in between treatment sessions and during follow-up included: Moisture Exposure Burn Ointment (MEBO) for the first three days after each session, later a sunscreen SPF 50+, topical facial moisturizer and gentle facial cleanser.

5.4.3. Rescue Medication

In case of herpes simplex eruption, valacyclovir was administered at a dose of 2 grams twice daily for 1 day. Therapy had to be initiated at the earliest symptom of herpes (e.g., tingling, itching, or burning).

5.5. Intervention

5.5.1. General for all treatment sessions

On the day of each session, treatment areas were cleaned with a mild cleanser and normal saline solution-. 10.5% lidocaine cream was applied 60 minutes before the treatment for pain relief. PRP was prepared by using a two-stage centrifugation process. Whole blood sample (18 ml) was drawn from peripheral venous blood of the patient and collected in two sterile tubes (Juvederm PERLA's Tube, Juvederm Cosmétiques, Bulgaria); each containing 1 ml acid-citrate dextrose A (ACD-A) anticoagulant. The tubes were centrifuged in a centrifugal apparatus; first at 500 g for 10 min, followed by separation of the upper 3 ml which will yield Platelet-Poor Plasma (PPP). A second spin was centrifuged at 1000 g for 7 minutes, followed by separation of 2 ml from the middle-squeezed portion of the tube to yield platelet-rich plasma (PRP). Calcium gluconate was added at a ratio of 1:9 (calcium gluconate/PRP) 5 minutes prior to treatment to activate platelets.

5.5.2. Intervention for 1st treatment session

In the first session, 3 spots were marked as the most marked 3 scars according to an agreement between the treating doctor and patients. Patients were treated using combined fractional ablative CO_2 laser (10,600 nm, Smartxidot^2; DEKA, M.E.L.A., Florence, Italy) only over spots with post-acne scars, except for one predetermined control spot, using D-pulse mode, stack 5, density 20% and dwell time 0.4 to 0.8 ms for all spots in this session, then erbium-doped laser (1550 nm, Fraxel II, Solta medical) was applied for treating the entire face using density 20% and energy 50 mJ immediately after fractional ablative carbon dioxide laser, except the same single control scar. Two ml of activated PRP was injected into the scar using a 22-gauge needle at dermal level at all fractional carbon dioxide laser spots immediately after each combined laser session except for two of the predetermined spots; one of which was the untreated control, the second was injected by Normal Saline Solution (NSS) after combined lasers treatment. 3 punch biopsies of 2 mm were taken 20 minutes after this laser session from 3 spots: the control spot and 2 lasers treatment spots (a. control with neither lasers nor PRP injection, b. post combined lasers and NSS, c. post combined lasers and PRP injection). Sutures were carried out with 5.0 nylon (one point).

5.5.3. Intervention for subsequent treatment sessions

The patients were scheduled for 3 subsequent sessions with a 4-week interval. Combined lasers were applied followed by PRP injections for all the carbon dioxide laser spots including the punch excision spots in the 1st session. Stack 5 ensured the deepest localized laser effect and was chosen for the first session; stack was lowered in subsequent sessions to reach more superficial levels.

5.6. Evaluation

5.6.1. Early Subjective clinical evaluation

Subjective evaluation consists of a new Patient-Oriented Tool questionnaire (**Table 9 & 10**) given to the patients for assessing their atrophic acne scarring through two tools; a) Assessment of Acne Scar Appearance (SCARS) and b) Acne Scar Quality of Life questionnaire (FASQoL).(29) Patients recorded their responses before the 1st session and on the date of 2nd session after 1 month without seeing their earlier answers. The relative percentual difference in scores (100 x [baseline – 1st session]/baseline) was calculated for each patient to assess early subjective clinical improvement. An ordinal grading scale was used (< 25%= poor; 25–50% = slightly satisfied, 51–75% = satisfied; and ≥75%= highly satisfied).

5.6.2. Final clinical Subjective evaluation

Three months after the 4th session, a final self-assessment through an Atrophic Acne Scars VAS was conducted by each patient on for comparison to the similar previous assessment. Similarly, relative percentual difference in scores (100 x [baseline – final]/baseline) and the same ordinal grading scale was calculated for each patient to assess final subjective clinical improvement.

Table (9): SCARS patient-reported outcome tool. Adapted from (29)

Assessment of Acne Scar Appearance and Severity: Part 1

Please remove all make-up and facial jewelry/piercings, and tie back any loose hair covering your face before answering this questionnaire.

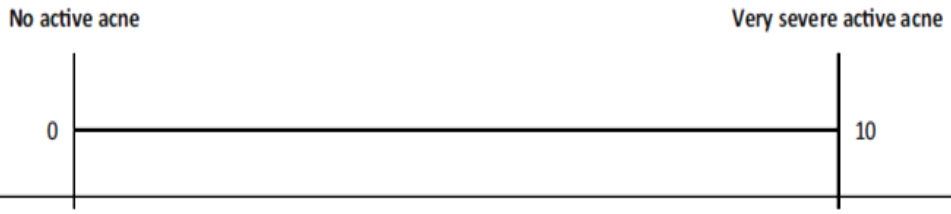
Please begin by answering the questions below. These questions will help you understand the difference between active acne and atrophic acne scars.

a) Active acne includes zits, breakouts, pimples, whiteheads, and blackheads.

When looking at your face in the mirror right now, do you see **zits, breakouts, pimples, whiteheads, or blackheads?**

Yes / No (please circle one)

If yes, please **rate the severity** of the **zits, breakouts, pimples, whiteheads, or blackheads** on your face by putting a vertical line in the place that best describes your acne.



The following question asks you about atrophic acne scars only. Please do not consider active acne (zits, breakouts, pimples, whiteheads, or blackheads), scabs, or flat red or dark marks.

b) Atrophic acne scars are indents or holes in the skin from previous active acne (not from injury, scratching or picking).

When looking at your face in the mirror right now, do you see **indents or holes** in the skin from previous active acne?

Yes / No (please circle one)

If you answered "No" for this question, you have completed this questionnaire. Thank you for your time.

If yes, please **rate the severity** of the **indents or holes** on your face by putting a vertical line in the place that best describes your scars.

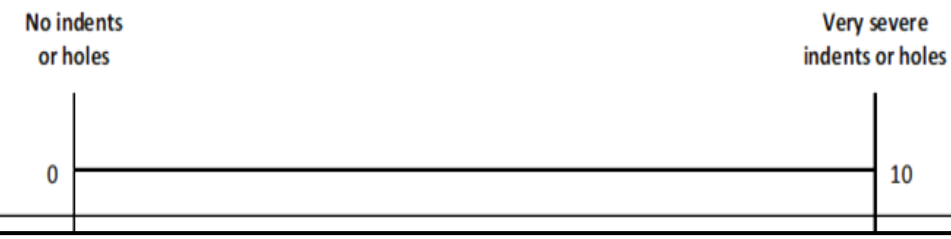


Table (9) continued: SCARS patient-reported outcome tool. Adapted from (29)

Assessment of Acne Scar Appearance and Severity: Part 2

The following questions ask you about atrophic acne scars only. Remember, atrophic acne scars are indents or holes in the skin from previous active acne. Please do not consider active acne (zits, breakouts, pimples, whiteheads, or blackheads), scabs, or flat red or dark marks.

Please read each question, then look in the mirror and think about the indents or holes on your whole face, and then answer the question. Please mark an "X" in the box (☒) that best describes how the indents or holes on your face look RIGHT NOW.

There are no right or wrong answers to these questions. If you want to change your answer, please cross out your original answer and mark an "X" in the correct box.

Please choose only one response.

1. **When looking at your face in the mirror right now, how much of your face looks covered by indents or holes?**

Almost none of my face	A little of my face	Some of my face	A lot of my face	Almost all of my face
☐	☐	☐	☐	☐

2. **When looking at your face in the mirror right now, how small or large do the individual indents or holes look?**

Very small	Small	Moderate	Large	Very large
☐	☐	☐	☐	☐

3. **When looking at your face in the mirror right now, how many indents or holes do you see?**

Very few indents or holes	A few indents or holes	Some indents or holes	Quite a lot of indents or holes	Many indents or holes
☐	☐	☐	☐	☐

4. **When looking at your face in the mirror right now, how deep do the individual indents or holes look?**

Not at all deep	A little deep	Moderately deep	Very deep	Extremely deep
☐	☐	☐	☐	☐

5. **When looking at your face in the mirror right now, how visible are the indents or holes to you?**

Not at all visible	A little visible	Moderately visible	Very visible	Extremely visible
☐	☐	☐	☐	☐

Table (10): FASQoL patient-reported outcome tool. Adapted from (29)

Acne Scar Quality of Life Questionnaire

The following questions ask you about how the atrophic acne scars on your face impact your daily life. Atrophic acne scars are indents or holes in the skin from previous active acne (not from injury, scratching or picking).

When answering these questions, please do not consider any of the following:

- **Zits, breakouts, pimples, whiteheads, or blackheads on the face;**
- **Acne scabs (dry, rough protective crusts that form over zits or pimples before indents or holes develop); or**
- **Flat red or dark marks.**

Please mark an "X" in the box (☒) that best describes the impact that the indents or holes on your face had on you over the PAST 7 DAYS.

There are no right or wrong answers to these questions. If you want to change your answer, please cross out your original answer and mark an "X" in the correct box. Please choose only one response.

Table (**10**) continued. Adapted from (29)

1. Over the past 7 days, have you felt self-conscious when you were with people because of the indents or holes on your face?

Not at all self-conscious	A little self-conscious	Somewhat self-conscious	Very self-conscious	Extremely self-conscious
☐	☐	☐	☐	☐

2. Over the past 7 days, have you felt less attractive because of the indents or holes on your face?

I did not feel less attractive	A little less attractive	Somewhat less attractive	Much less attractive	Extremely less attractive
☐	☐	☐	☐	☐

3. Over the past 7 days, have you felt annoyed because of the indents or holes on your face?

Not at all annoyed	A little annoyed	Somewhat annoyed	Very annoyed	Extremely annoyed
☐	☐	☐	☐	☐

4. Over the past 7 days, have you felt worried that the indents or holes on your face won't go away?

Not at all worried	A little worried	Somewhat worried	Very worried	Extremely worried
☐	☐	☐	☐	☐

5. Over the past 7 days, have you felt sad because of the indents or holes on your face?

Not at all sad	A little sad	Somewhat sad	Very sad	Extremely sad
☐	☐	☐	☐	☐

6. Over the past 7 days, have you felt upset by negative comments from others because of the indents or holes on your face?

Not at all upset	A little upset	Somewhat upset	Very upset	Extremely upset
☐	☐	☐	☐	☐

7. Over the past 7 days, have the indents or holes on your face made you avoid going out with friends or family?

Not at all	A little	Somewhat	Very much	Extremely
☐	☐	☐	☐	☐

8. Over the past 7 days, have you felt bothered by having to hide the indents or holes on your face?

Not at all bothered	A little bothered	Somewhat bothered	Very bothered	Extremely bothered
☐	☐	☐	☐	☐

9. Over the past 7 days, have the indents or holes on your face affected your relationships with others (for example, friends, family, romantic relationships/partner)?

Not at all	A little	Somewhat	Very	Extremely
☐	☐	☐	☐	☐

10. Over the past 7 days, have the indents or holes on your face affected your participation at work or school?

Not at all	A little	Somewhat	Very	Extremely
☐	☐	☐	☐	☐

5.6.3. Objective clinical evaluation

Photographs of the patient's face in 3 positions (front, left side, right side) were obtained using identical camera settings, and lighting at baseline and 3 months after the 4th treatment. Objective clinical assessments were made by four physicians blindly evaluating 2 sets (each one composed of 3 pictures) of photos taken before and after the procedure, by scoring post-acne scars severity on a (0-10) scale for the 2 sets of each patient, shown in random nonchronological order. The four assessors were not informed as to the study design. The relative percentual difference in scores (100 x [baseline – final]/baseline) was calculated in each patient for the four assessors to assess objective clinical improvement. For inter-rater agreement, an ordinal grading scale was used (< 25%= poor; 25–50% = slightly satisfied, 51–75% = satisfied; and ≥75%= highly satisfied).

5.6.4. Histological Evaluation

Biopsies were immediately fixed by immersion in a 4% formaldehyde solution in sodium phosphate pH 7.4 buffer. Skin biopsies were then dehydrated through graded ethanols, cleared in xylene, and embedded in paraffin. Embedded samples were microtome sectioned (7 μm), stained and processed with **1)** Haematoxylin and Eosin for morphological analysis; **2)** Picrosirius Red 0.1 % solution for COL staining and enhancing birefringence; **3)** Indirect immunoperoxidase method(145) with some modifications for the immunohistochemical detection of COL I, COL III and MMP-2.

5.6.4.1. Picrosirius Red Stain

Adjacent sections, with the same thickness (7 μm) for all specimens (i.e. 3 specimens per patient), were stained in the same staining solution at the same time, with Sirius red (Sirius red F3B, Sigma-Aldrich, St Louis, MO, USA) solution 0.1% in a saturated aqueous solution of Picric acid for 1 h, at room temperature, followed by an acidified water washing (5 ml acetic acid in 1-liter water). To identify mature and newly formed COL and therefore to get evidence of possible regeneration and remodeling of the connective tissue, a Zeiss Axioplan microscope (Carl Zeiss, Oberkochen, Germany) equipped with suitable filters for circularly polarized

microscopy, was used. The filters were aligned so that the background in the field of view was as dark as possible (i.e. filters were crossed) and birefringent structures COL I and COL III could be clearly identifiable. Morphometric computerized analysis of control and treated biopsies was performed using ImageJ software version 1.49h (Waine Rasband, National Institutes of Health, Bethesda, USA) on microscopic digital images recorded with a Nikon DS-Fi2 high definition 5-megapixel color CCD camera head (Nikon, Tokyo, Japan).

Under circularly polarized microscopy, to define the proportions of differently coloured COL fibers, digital images from sections were segmented into two-colour threshold bands (Green/Yellow G/Y and Red/Orange R/O) in the color Hue, Saturation and Brightness (HSB) space. Suitable HSB values have been defined to create masks that could be exactly superimposed on the images for extracting the structures of interest for measurements. The ImageJ software program will accurately assess the number of pixels for each of the two-colour threshold bands. The steps used in this study are detailed in (Table **11**).

Table (**11**): ImageJ steps used for morphometric evaluation

-File/Open. Choose the image
-Image/Adjust/Color Threshold
Color Threshold:
-Hue: 19-170 (Yellow/Green channel, COL III)
0-18 (Red/Orange channel, COL I)
0-255 (reference area of the whole COL I + COL III)
-Saturation: Auto (0 - 255)
-Brightness: Auto
-Process/Binary/Make binary
-Define width frame (µm): click left and drag right the extremes of frame
-Analyze/Set scale (on Set scale window, control Known distance as frame width).
Mark Global
-Analyze/Analyze particles (Area μm^2).
In the Summary window obtained, the numeric value in the column Total Area was reported in Excel sheet, in the box related to the specific biopsy.

The same parameters defining threshold bands of HSB were applied to all the images without any modification, permitting a quantitative and comparative evaluation of both **mature** (COL **I**) and **newly formed** (COL **III**) COL fibers, in terms of areas occupied by COL I and COL III respectively, referred as percentages respect to the whole COL content only (i.e. the background and sebaceous gland

areas on the screen were not included). This was possible because Picrosirius Red staining is specific for COL, enhancing its natural birefringence, and no other structures in the skin were birefringent.

5.6.4.2. Immunohistochemical staining

Rehydrated sections were incubated serially with the following solutions and treatments: 1) 3% hydrogen peroxide for 30 min to remove endogenous peroxidase activity; 2) pepsin (P-7125 Sigma-Aldrich, St Louis, MO, USA) digestion (0.1% in 10 mM HCl at 37°C for 30 min; 3) normal goat serum (Sigma), diluted 1:20 for 30 min to reduce background staining; 4) primary antibodies to COL I (polyclonal), COL III (monoclonal) and MMP-2 (monoclonal) (Novus Biologicals, Littleton, CO, USA) at dilutions of 1:200, overnight at 4°C; 5) Polymer HRP anti-Mouse/Rabbit IgG (RTU), for 30 min at room temperature and DAB chromogen for 5 min at room temperature (Detection System by GBI Labs, Bothell, WA98021, USA).

Specificity tests of the immunohistochemical reactions were performed, with the omission of the first antibody layer and substitution of a non-immune serum for the specific primary antibody. Immunostained sections were finally rehydrated, mounted, and observed using a Zeiss Axioplan microscope.

5.7. Statistical Analysis

After receiving evaluation sheets from the 4 blind assessors and the histopathologist, the database was completed, and the randomization codes have been unveiled. Statistical analysis was carried out with STATA software (version 14.2; StataCorp LLC, Texas, USA).

5.7.1. General considerations

The main objective of the study was to evaluate the efficacy of Combined Fractional LASERs Resurfacing with Platelets Rich Plasma (PRP) for treating Post-acne atrophic scarring.

The level of statistical significance of the different bilateral tests of all the analyses will be 5%.

5.7.2. Deviations from the protocol

A deviation was to be considered important when it could exert a significant bias in the calculation of the treatment effect based on the main criterion of effectiveness. Patients with significant deviations were excluded from the set of data analysed.

The set of data analysed corresponds to all patients who completed the four treatment sessions of the study.

5.7.3. Primary Outcomes

The first criterion of successful treatment is the minimization of Post-acne scars self-assessment scores from the basal visit treatment to week 4 visit. The second criterion will be evaluated by comparing the 4 blind assessors' quantitative assessments of the digital photographs of week 24 with the baseline photographs, where each assessor will independently evaluate 2 sets of 3 photographs without being informed the timeline for each set. A third criterion will be the final patients' VAS score at week 24.

Patients who discontinued treatment sessions for more than 2 months between visits from week 4-12 were classified as withdrawn.

For patients who took a prohibited concomitant treatment before the week 12 visit, it was

up to the validation committee to decide on their being classified as withdrawn.

The significance of the change in these criteria was analysed with a Wilcoxon signed-rank test. For measuring inter-rater agreement, four Ordinal Categories were created from ratios of change in Atrophic Acne Scars VAS for all assessors and patients as follows: **Highly Satisfied** (>75%), **Satisfied** (50–75%), **Slightly Satisfied** (25–50%), and **Poor** (<25%) to allow Kappa Statistics evaluation.

5.7.4. Secondary Outcomes

The first secondary outcome evaluation was the ImageJ computerized quantitative calculation for the COL I and COL III staining by PSR under circularly polarized microscopy after the nullification of the background. The importance of background omission is to maximize the accuracy of the ratio of COL I or III to the surrounding connective tissue in specimen only rather than calculation of ratio to the whole field. Repeated measures of ANOVA test were used for correlations of histological results among different groups. Full Descriptive statistics are provided for each COL type by treatment group.

The second secondary outcome evaluation was the histopathologist semi-quantitative blind assessment for Immunohistochemical staining of COL I, COL III, and MMP-2 for the 3 biopsies taken from each patient. For the 288 immunohistochemical stained slides, a 5-grade scale for immunoreactivity was used ("**0**" for absent immunoreactivity, "**1**" for scarce, "**2**" for weak, "**3**" for moderate, "**4**" for intense). Friedman's test was used for correlations of immunoreactivity results among different groups.

5.7.5. Intermediate analysis and Data monitoring

No intermediate analysis was foreseen. In the absence of any relevant systemic exposure in the study, no safety problems were expected. Thus, in this study, there was no independent data monitoring committee or data and security monitoring board.

5.8. Ethical aspects

5.8.1. General Considerations

The study was carried out following the international ethical recommendations for research and clinical trials in humans. The researcher was responsible for ensuring that the clinical trial was carried out in accordance with the rules contained in the Helsinki Declaration and following the recommendations of the Spanish Ministry of Health in clinical trials.

Before including any subject in the subject, the Ethical Committee of Cosmetic Surgery Clinic Kuwait approved the protocol of the study, the information that was to be given to the subject and the informed consent model to be used.

The study was developed in accordance with the standards of Good Clinical Practice (GCP), as described in the ICH Harmonized Tripartite Rules for Good Clinical Practice 1996.

Since the study took place as part of the Autonomous University of Barcelona PhD program, European medicolegal requirements were fulfilled for the informed consent., as follows.

5.8.2. Information sheet for the patient and consent

It is the responsibility of the investigator to obtain the informed consent of the patients. The patients cannot participate in any specific procedure of the study before providing signed informed consent.

Before the beginning of the trial, and before obtaining the informed consent, the investigator will fully explain to the patients the objectives, methods and potential risks of the study and any inconvenience that it may cause. This will be carried out in an understandable language.

The patients will have time to decide on their participation in the study and have the opportunity to ask questions.

The information provided should include:

• Explanation that the trial involves an experimental procedure.

• Explanation of the objective of the study.

• Description of the procedures to follow, including the invasive procedure, duration of participation, and approximate number of subjects that will participate in the essay.

• Responsibilities of the patients.

• Risks and inconveniences reasonably for the subject and planned measures of solution.

• Description of benefits for the patients / society.

• Availability of alternative treatments with their potential risks and benefits.

• Compensation to the subjects: coverage of the risks, medical treatment of the possible damages, economic compensation.

• Knowledge of any additional cost for the subject that may be derived from your participation in the investigation.

• Conditions of participation: Consent expressed according to your free will, right to abandon the trial at any time, right to refuse to participate without prejudice to the subject.

• Explanation that the identity of the subject is confidential but that the stories can be reviewed by the test monitor, the auditors and may be made aware of the health authorities.

• Statement that new relevant findings will be made available to the subject.

• Identification of who and to which service they can go to obtain answers regarding any aspect of the trial or the rights of the subject (name and telephone number).

• In case the patient is a female, if deemed necessary, the investigator will provide additional information to prevent the possibility of a pregnancy during the process of selection, development and follow-up of a trial.

The Information Sheet Model for the Patients and Consent Form is in the

attached sheet. (See Annex)

5.8.3. Evaluation of the expected benefits and risks of the trial

With respect to the evaluation of the benefits in this study, a dermatological evaluation of all patients will be maintained, so that patients who experience excessive inflammatory acne outbreak, ulceration or disfigurement, will be given other options of management with systemic therapy e.g. oral retinoids. Such a procedure will be also confirmed through patients' records in their SCARS and FASQoL tools 4 weeks after the 1st session.

Regarding the foreseeable risks, no serious side effects have been reported to date or that put the patient at risk. The effects that may occur are secondary to photosensitivity, which will be evaluated at each visit to avoid risk, the patients will be instructed about possible side effects and what they can do to prevent them or to be in close contact with them before any doubt.

It is possible that patients do not benefit from participating in this study, however, the results of the study will determine whether LASERs combination with PRP is effective for early enhancement of improvement in post-acne scars management.

5.8.4. Confidentiality of the data

To preserve the confidentiality of the personal data of the subjects, only the principal investigator, his collaborators, and the technical personnel that participates in the study will have access to the identity of the subjects. For the same reason, full filiation and written consent data will be stored in the archive of the investigator's center.

As regards the confidentiality of the data of the study, the provisions of the Organic Law 15/1999 of December 13, on "Protection of Personal Data" will be followed. In accordance with Law 15/1999 on the Protection of Personal Data, personal data that are required of the patients are those necessary to cover the objectives of this study. In any of the reports of the study no names will appear, and identities will not be revealed to any person except to fulfil the purposes of the study, and in the case of medical urgency or legal requirement.

Any personally identifiable information that may be identifiable will be preserved and processed by computer media in safety conditions by the study's researchers. Access to said information will be restricted and always carried out under conditions of confidentiality. The results of the study may be communicated to the health authorities and, eventually, to the scientific community, without the identity of the participating subjects being in any case.

In accordance with the law in force, the subject participant in the study has the right to access their personal data and, if justified, has the right to request rectification or cancellation.

Patient data collected in the Data Collection Notebook during the study must be documented anonymously and dissociated, linking to a code (patient number), so that only the researcher can associate such data with an identified person or identifiable.

The database generated by the study will not contain any identification of the patient, more than a numerical code for which it will not be possible to reveal their identity. The information collected in the study will always be treated as grouped data and never as an individual or personal data, thus maintaining anonymity and confidentiality.

5.9. Limitations:

In our study, there were a few ethical and technical limitations. Consent for taking biopsies from the face is generally difficult to be accepted by patients, however, we limited our biopsies to the 3 most marked ("ugly" in the patient's opinion) deep scars which required punch excisions as a treatment modality. Also, it would have been better to have obtained a biopsy specimen at the end of treatment for comparison, but it would not have been ethically acceptable because of the residual scar line, whereas when taken earlier on the first session, the subsequent sessions will be effective in improving any residual scars. Technically, formaldehyde preserved biopsies are not the best for immunolabelling techniques if compared to cryopreserved biopsies. This is due to the partial loss of antigenicity of epitopes. On the other hand, cryopreserved biopsies would not allow the simultaneous picrosirius red stain for all biopsies, which was needed for comparative accuracy; moreover, higher costs of cryopreservation and not being easily available in the private center added another limitation. Thus, we decided to perform immunohistochemical staining for COL type I, COL type III, and MMP-2, which are the secondary outcomes on a histopathological basis, after formaldehyde preservation.

6. Results

Results

6.1. Patients' Demographic Data

A total of 32 patients were included in this study. In all patients, 3 biopsies were taken. Their clinical characteristics and study participation details are summarized in Table 12.

Table 12: Complete basal characteristics and interventions of the patients included in the study.

Serial	Gender	Skin Type	Age	Active Acne lesions	Control Biopsy (Wk 0)	LASER + NSS Biopsy (Wk 0)	LASER + PRP Biopsy (Wk 0)	Before Patient's Quest. (Wk 0)	After Patient's Quest. (Wk 0)	Final Patient's Score (Wk 24)	Blind Assessors Before Score	Blind Assessors After Score
1	M	III	32	×	✓	✓	✓	✓	✓	✓	✓	✓
2	M	IV	34	×	✓	✓	✓	✓	✓	✓	✓	✓
3	F	III	29	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	F	IV	19	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	F	IV	41	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	M	III	27	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	M	V	23	×	✓	✓	✓	✓	✓	✓	✓	✓
8	M	III	33	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	M	IV	31	×	×	×	×	×	×	×	×	×
1û	F	IV	35	×	✓	✓	✓	✓	✓	✓	✓	✓
11	F	IV	33	×	✓	✓	✓	✓	✓	✓	✓	✓
12	F	III	38	✓	✓	✓	✓	✓	✓	✓	✓	✓
13	F	III	20	✓	✓	✓	✓	✓	✓	✓	✓	✓
14	M	III	40	✓	✓	✓	✓	✓	✓	✓	✓	✓
15	M	IV	38	✓	✓	✓	✓	✓	✓	✓	✓	✓
16	F	III	30	✓	✓	✓	✓	✓	✓	✓	✓	✓
17	M	IV	26	✓	✓	✓	✓	✓	✓	✓	✓	✓
18	F	IV	38	✓	✓	✓	✓	✓	✓	✓	✓	✓
19	F	III	33	✓	✓	✓	✓	✓	✓	✓	✓	✓
2û	F	III	42	×	✓	✓	✓	✓	✓	✓	✓	✓
21	F	IV	46	×	✓	✓	✓	✓	✓	✓	✓	✓
22	F	IV	37	✓	✓	✓	✓	✓	✓	✓	✓	✓
23	M	III	28	×	✓	✓	✓	✓	✓	✓	✓	✓
24	M	III	28	×	✓	✓	✓	✓	✓	✓	✓	✓
25	F	IV	24	✓	✓	✓	✓	✓	✓	✓	✓	✓
26	M	III	19	×	✓	✓	✓	✓	✓	✓	✓	✓
27	F	IV	36	✓	✓	✓	✓	✓	✓	✓	✓	✓
28	F	IV	24	✓	✓	✓	✓	✓	✓	✓	✓	✓
29	F	III	34	×	✓	✓	✓	✓	✓	✓	✓	✓
3û	F	IV	29	✓	✓	✓	✓	✓	✓	✓	✓	✓
31	M	III	32	×	✓	✓	✓	✓	✓	✓	✓	✓
32	F	III	33	✓	✓	✓	✓	✓	✓	✓	✓	✓

Only one patient was withdrawn from the study after the first session because he did not attend the second session.

The mean age at inclusion was 31.64 years ± 6.86 with a normal distribution as shown in Figure 18. The patients were 19 females (61.3%) vs. 12 males (38.7%) (Figure 19).

Figure 18: Histogram for patients' age

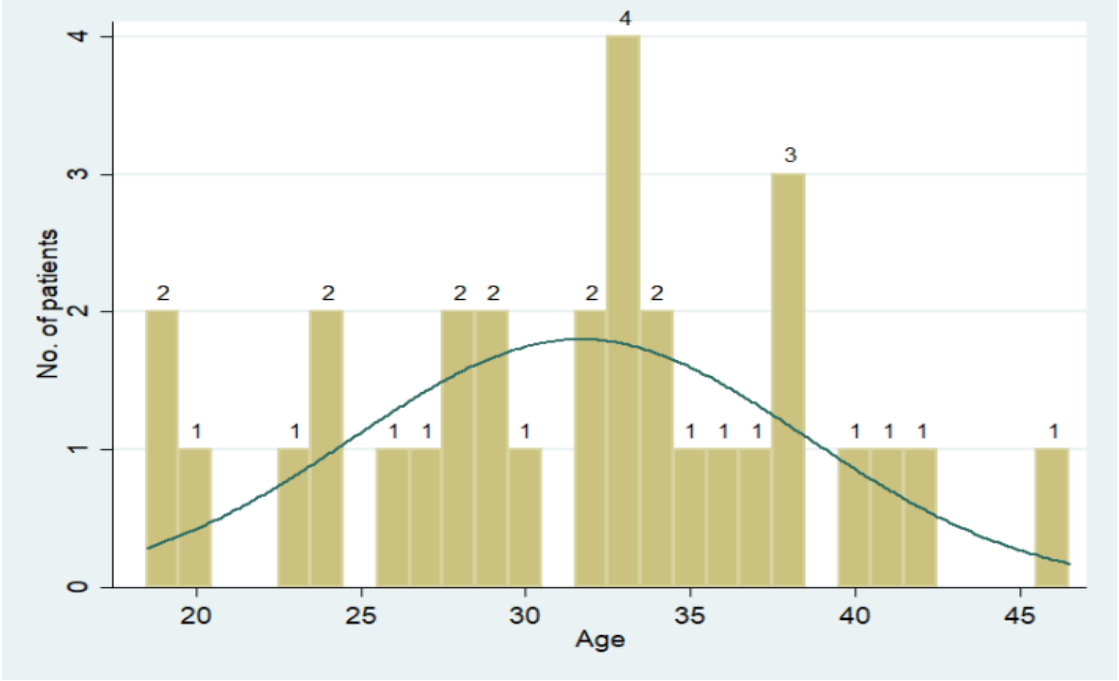
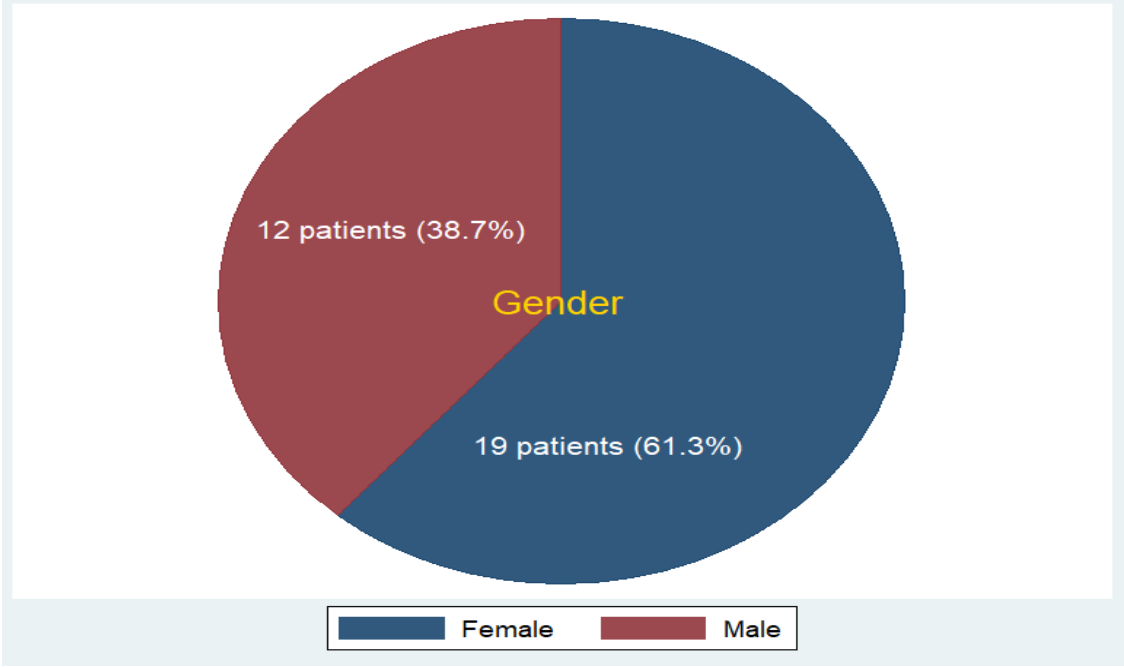
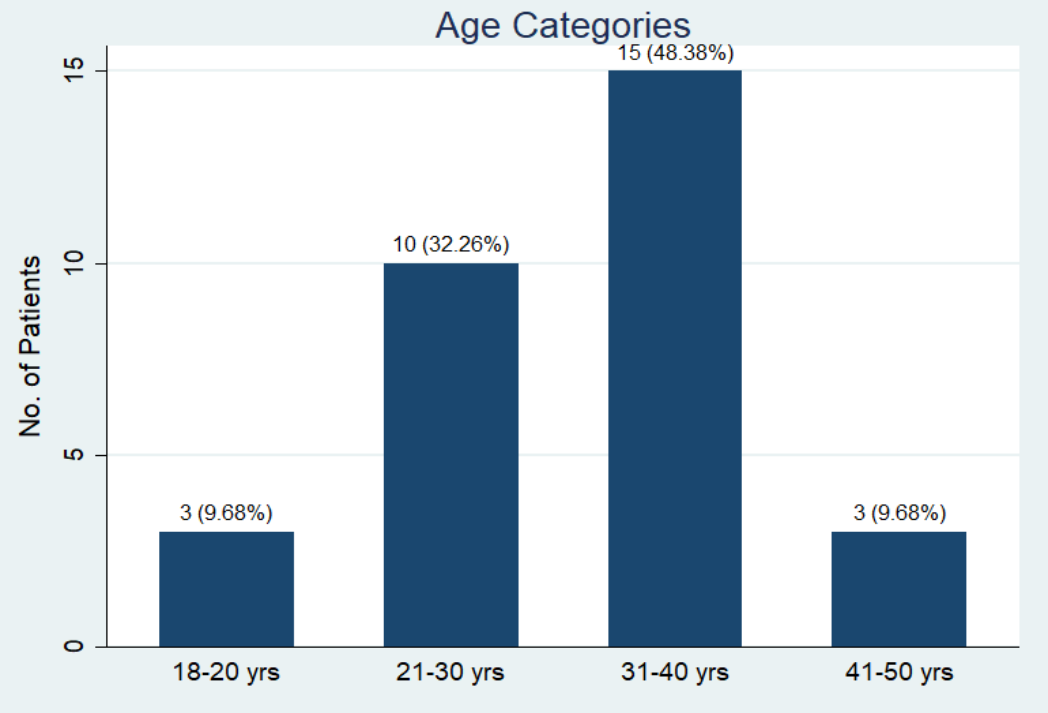


Figure 19: Gender Pie Chart



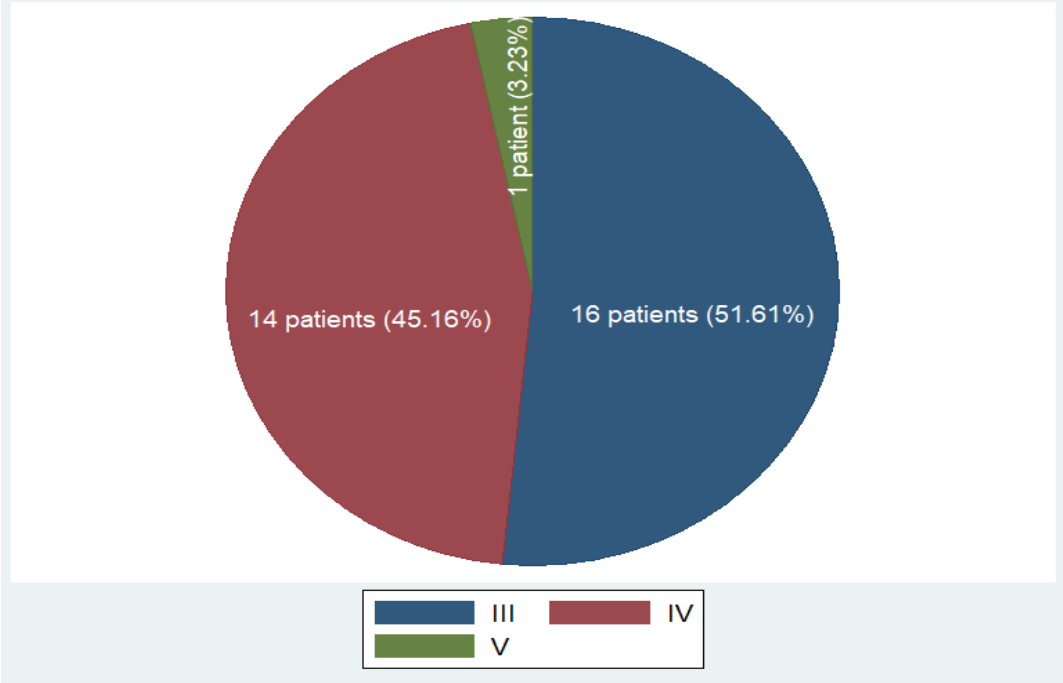
Patients were classified into 4 age categories: 18-20 yrs (9.68%), 20-30 yrs (32.26%), 30-40 yrs (48.38%), 40-50 yrs (9.68%) as shown in (Figure 20).

Figure 20: Distribution of patients among age categories



Skin Types (III-IV) were included in our study as shown in (Figure 21)

Figure 21: Skin types for participants



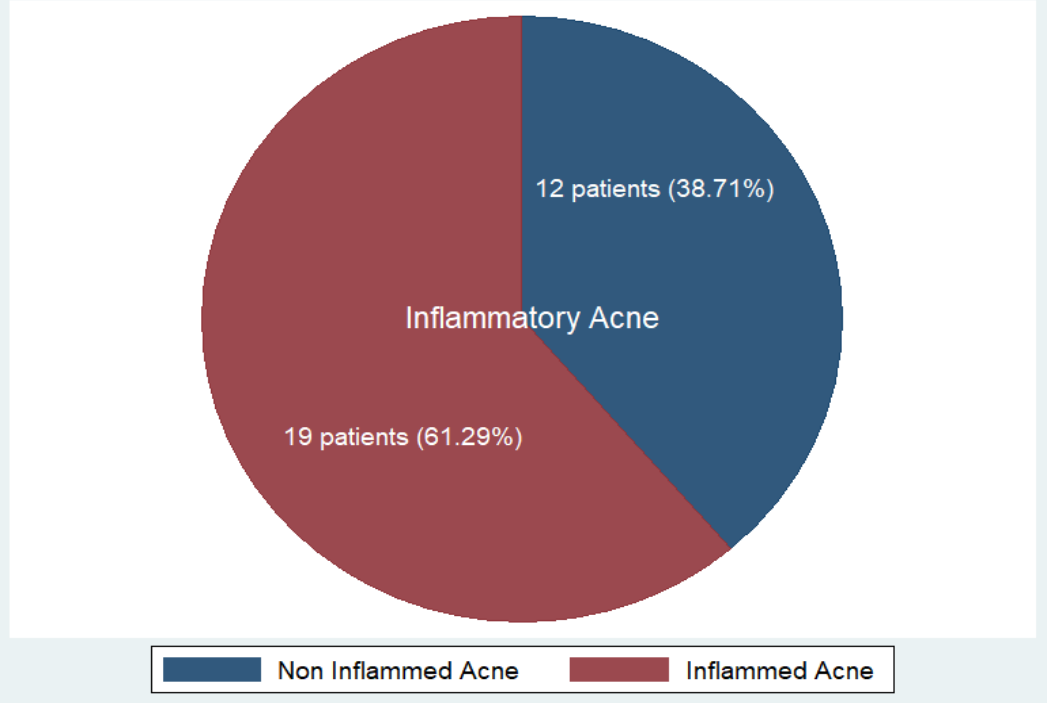
The basal characteristics for the 31 patients included in the study in terms of Skin Type and Age Categories are shown in the following table (13)

Table 13: Basal characteristics of the patients (Age categories vs. Skin types)

	Age Category				
Skin Type	18-20 yrs	20-30 yrs	30-40 yrs	40-50 yrs	Total
III	2	5	8	1	16
IV	1	4	7	2	14
V	0	1	0	0	1
Total	3	10	15	3	31

Our study included 19 patients with mild inflammatory acne lesions (61.29%) and 12 patients without inflammatory acne lesions (38.71%) as shown in (Figure 22).

Figure 22: Active and Non-active acne Pie Chart



6.2. Evaluation of Primary Outcome

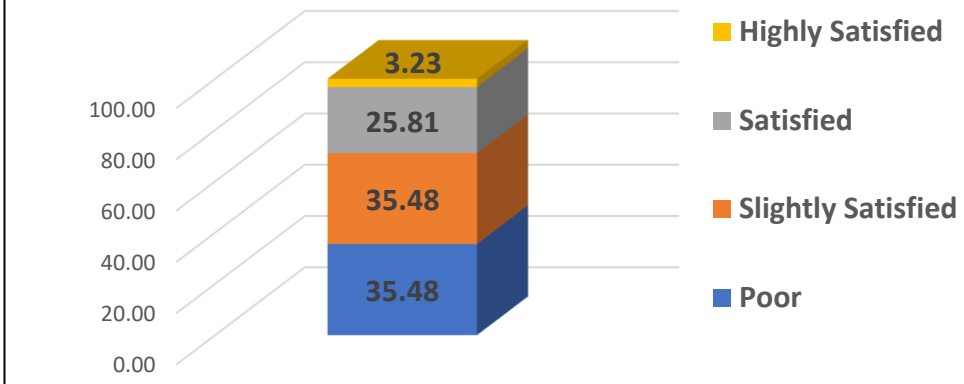
The primary outcome evaluation was done at two points through the course of the study; the first was at week 4 by a Patient-Oriented Tool questionnaire to assess early clinical improvement and the second was at week 24 through independent blind assessors' evaluation and patients' self-assessment for post-acne scars severity.

6.2.1. Early Clinical Improvement (week 4):

The Patient-Oriented Tool consists of Part 1 subdivided into a) Active Acne Visual Analogue Scale and b) Atrophic Acne Scar Visual Analogue Scale, and Part 2, consisting of 5 questions with 5 ordinal answers, and the FASQoL for Acne Scar Quality of Life, consisting of 10 questions with 5 ordinal answers.

In the 19 patients with active acne lesions, Part 1a; Active Acne Scars VAS score, pre-treatment values were significantly higher than posttreatment values after 1st session (5.05±2.52 vs 2.52±1.98, Wilcoxon, *P*=0.0003).
Similarly, in the whole cohort, Part 1b; Atrophic Acne Scars VAS score, pre-treatment values were significantly higher than posttreatment values (7.03±1.97 vs 4.61±1.94 Wilcoxon, P<0.0001).). Only 1 patient was highly satisfied, 8 patients were satisfied, 11 patients were slightly satisfied, and 11 patients were poorly satisfied (Figure 23).

Figure 23: Patients' VAS relative percentual difference after 1st session



Part 2; Atrophic Acne Scars Severity score is a 5-item instrument with one hypothesized domain that asks subjects to rate the severity of acne scars as seen in a mirror. Pre-treatment values were significantly higher than posttreatment values (12.25±3.29 vs 9±3.38 Wilcoxon, P<0.0001).

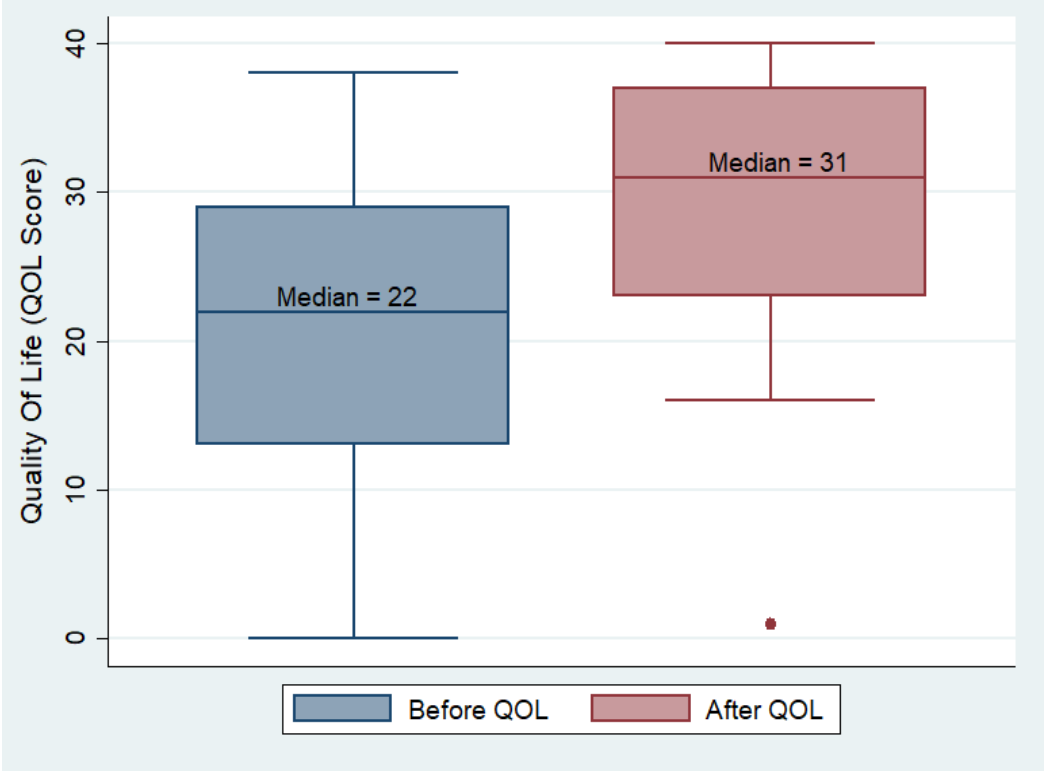
Table 14: Patient satisfaction and FASQoL after 1st session

Serial	Gender	Age	Patient Satisfcation After 1st session	FASQoL Before	FASQoL After	FASQoL Change
1	M	32	Poor	33	36	3
2	M	34	Slightly Satisfied	33	37	4
3	F	29	Poor	22	33	11
4	F	19	Slightly Satisfied	26	28	2
5	F	41	Satisfied	15	23	8
6	M	27	Slightly Satisfied	28	31	3
7	M	23	Slightly Satisfied	32	36	4
8	M	33	Poor	13	16	3
9	M	31	Withdrawn			
10	F	35	Slightly Satisfied	20	23	3
11	F	33	Satisfied	29	35	6
12	F	38	Slightly Satisfied	12	16	4
13	F	20	Satisfied	26	26	0
14	M	40	Poor	17	19	2
15	M	38	Satisfied	30	36	6
16	F	30	Satisfied	28	36	8
17	M	26	Poor	14	22	8
18	F	38	Slightly Satisfied	16	20	4
19	F	33	Poor	23	27	4
20	F	42	Highly Satisfied	24	40	16
21	F	46	Poor	24	38	14
22	F	37	Satisfied	13	30	17
23	M	28	Satisfied	8	39	31
24	M	28	Satisfied	0	40	40
25	F	24	Poor	0	1	1
26	M	19	Slightly Satisfied	38	39	1
27	F	36	Slightly Satisfied	21	27	6
28	F	24	Poor	11	28	17
29	F	34	Slightly Satisfied	11	22	11
30	F	29	Slightly Satisfied	12	39	27
31	M	32	Poor	30	31	1
32	F	33	Poor	31	38	7

FASQoL is a 10-item instrument with three domains assessing the impact of scars on emotions, social functioning, and work/school on 5-point rating scales with a recall period of the past 7 days. Unlike VAS scores and Part 2, FASQoL

pre-treatment values were significantly lower than posttreatment values (20.64±9.76 vs 29.41±9.14, Wilcoxon, P<0.0001). As sown in the Box and whisker (Figure 24), the medians of FASQoL were 22 and 31 for before and after, respectively.

Figure 24: Box and Whisker for FASQoL before and after 1st session



6.3. Evaluation of Secondary Outcomes

6.3.1. Final Clinical Improvement:

Final Clinical improvement was evaluated through calculating ordinal categories of the relative percentual difference for Atrophic Acne Scar score from the baseline at week 0 to follow-up visit at week 24 by four blind assessors and patients as shown in Table 15.

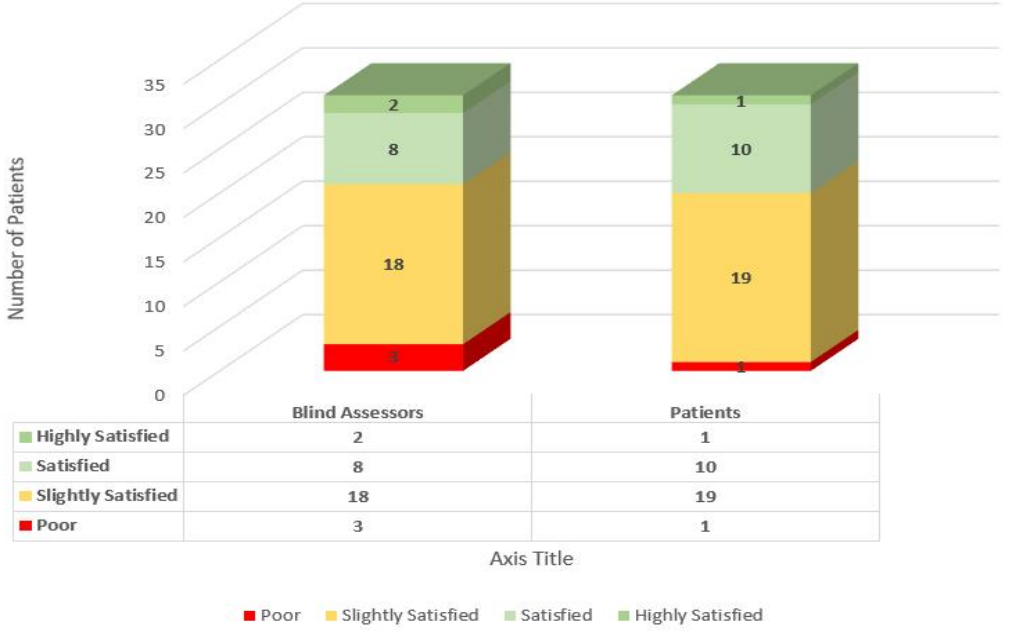
The mean blind assessors' pre-treatment values for Atrophic Acne Scars score were significantly higher than posttreatment values (5.5±1.81 vs 3.15±1.41 Wilcoxon, P<0.0001). The blinded assessors graded the final improvement of 2 patients as highly satisfactory, 8 patients as satisfactory, 18 patients as slightly satisfactory, and 3 patients were unsatisfactory (Figure 25).

Table 15: Final improvement ordinal categories

Serial	Gender	Age	Blind Assessors Final Improvement Category	Patients Self-assessment Final Improvement Category
1	M	32	Satisfied	Satisfied
2	M	34	Slightly Satisfied	Slightly Satisfied
3	F	29	Poor	Poor
4	F	19	Slightly Satisfied	Slightly Satisfied
5	F	41	Satisfied	Satisfied
6	M	27	Slightly Satisfied	Slightly Satisfied
7	M	23	Satisfied	Satisfied
8	M	33	Slightly Satisfied	Slightly Satisfied
9	M	31	Withdrawn	
10	F	35	Slightly Satisfied	Slightly Satisfied
11	F	33	Satisfied	Satisfied
12	F	38	Poor	Slightly Satisfied
13	F	20	Highly Satisfied	Highly Satisfied
14	M	40	Satisfied	Slightly Satisfied
15	M	38	Slightly Satisfied	Satisfied
16	F	30	Slightly Satisfied	Satisfied
17	M	26	Slightly Satisfied	Slightly Satisfied
18	F	38	Slightly Satisfied	Slightly Satisfied
19	F	33	Slightly Satisfied	Slightly Satisfied
20	F	42	Satisfied	Satisfied
21	F	46	Slightly Satisfied	Slightly Satisfied
22	F	37	Slightly Satisfied	Slightly Satisfied
23	M	28	Highly Satisfied	Satisfied
24	M	28	Slightly Satisfied	Slightly Satisfied
25	F	24	Slightly Satisfied	Slightly Satisfied
26	M	19	Satisfied	Slightly Satisfied
27	F	36	Poor	Satisfied
28	F	24	Slightly Satisfied	Slightly Satisfied
29	F	34	Satisfied	Slightly Satisfied
30	F	29	Slightly Satisfied	Satisfied
31	M	32	Slightly Satisfied	Slightly Satisfied
32	F	33	Slightly Satisfied	Slightly Satisfied

Similarly, the patients' self-assessment pre-treatment values for Atrophic Acne Scars score were significantly higher than posttreatment values (7.03±1.97 vs 3.94±1.36 Wilcoxon, P<0.0001). Only 1 patient was highly satisfied, 10 patients were satisfied, 19 patients were slightly satisfied, and 1 patient was poorly satisfied (Figure 25). Moreover, the post-treatment values after 1st session were significantly higher than final posttreatment values (4.61±1.94 vs 3.94±1.36 Wilcoxon, P<0.0001).

Figure 25: Comparison of final blind assessors vs. patients' evaluation



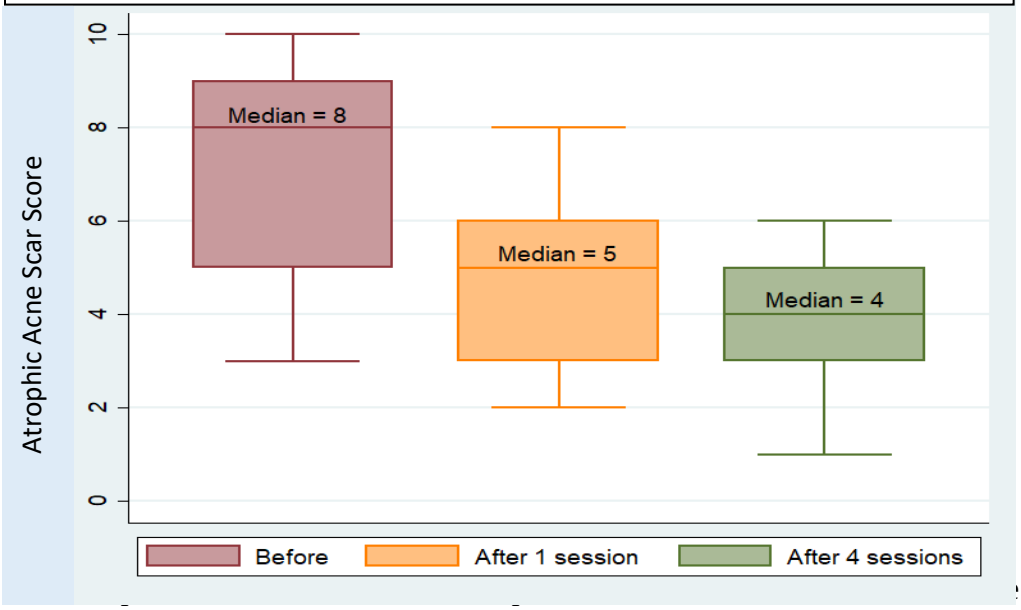
A representative example of the baseline to Week 24 comparison is shown in Figure **26**.

Figure 26
(A) A 20-year-old female (skin phototype III) with active acne and atrophic acne scars at baseline.
(B) Clinical appearance at week 24 after four combined laser treatments. (patient had a rhinoplasty surgery 2 months after 4th session).

Interrater agreement was tested using the kappa statistic.(146) Weighted kappa was 0.403, which meant that the four independent assessors had moderate strength of agreement.

Friedman's test (a nonparametric equivalent to the repeated measures analysis of variance) for Patients' self-assessment of Atrophic Acne Scars VAS scores at weeks 0, 4, and 24 showed that the median score among the three assessments differed significantly (Friedman = 69.92, p < 0.0001) as shown in Figure 27.

Figure 27: VAS Score across timeline (patients' self-assessment)



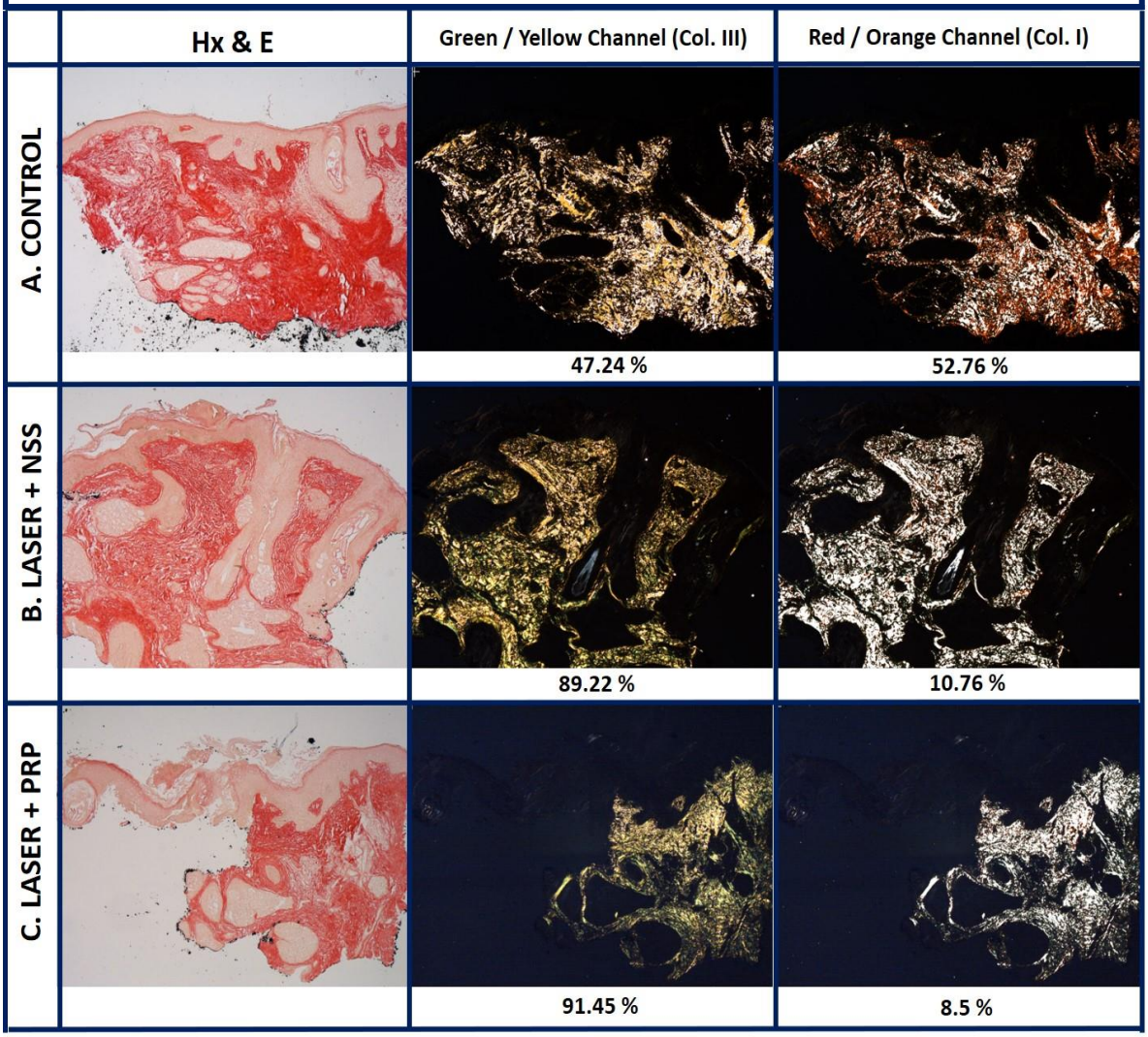
timeline of the study is shown in Figure 28.

Figure 28: Acne scars Score across timeline (patients and blind assessors)

6.3.2. Picrosirius Red Stain for COL III and COL I

Under circularly polarized microscopy, three digital images were captured from Control, Laser + NSS, and Laser +PRP biopsies from each patient. The quantitative assessment of COL III and COL I was done by ImageJ program computerized calculations of pixels for each of Yellow/Green and Red/Orange channels, respectively. (Figure 29)

Figure 29. Biopsies of Case no. 8 showing Hx & E, Picrosirius Red Stain Y/G and R/O Channels under circularly polarized microscopy for a) Control, b) LASER + NSS, and c) LASER + PRP



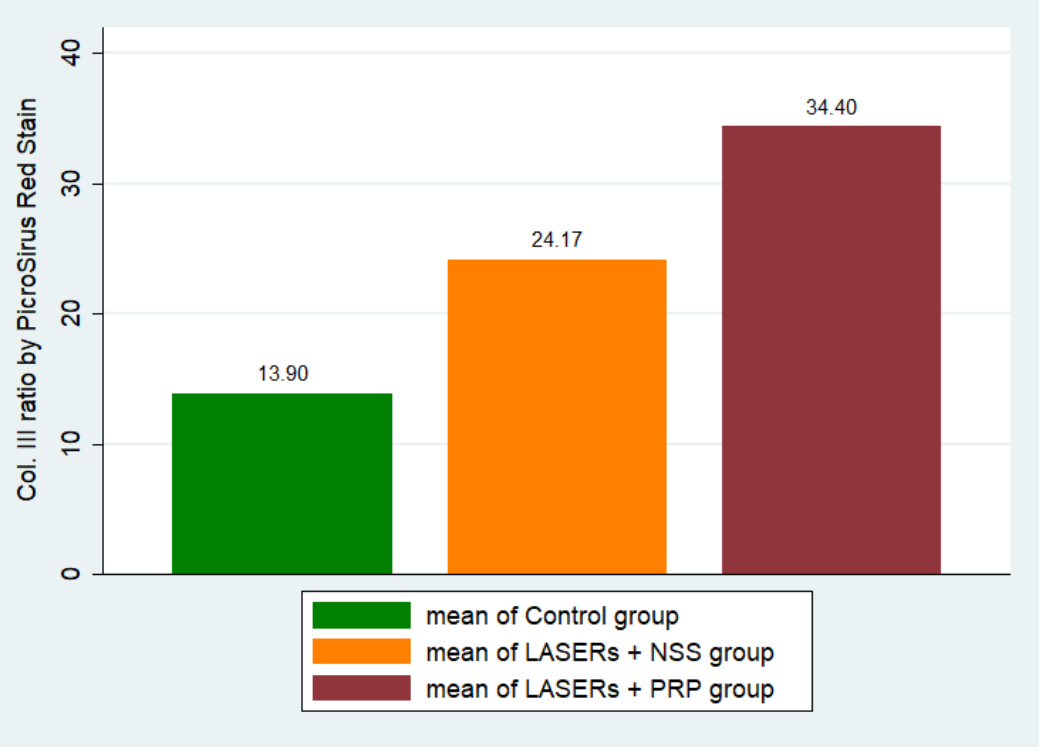
The variations among the three groups in mean ± SD of COL I, COL III and COL I/III ratio are shown in Table 16.

Table 16: Mean ± SD for Col. III & I in Control, Laser + NSS and Laser + PRP biopsies

	Control	Lasers + NSS	Lasers + PRP	P - value
COL III	13.9 ± 15.97	24.17 ± 21.67	34.41 ± 23.57	<0.0001
COL I	84.77 ± 15.6	75.05 ± 21.05	64.5 ± 23.13	<0.0001
COL I/III ratio	≃ 29.9: 1	≃ 7.4: 1	≃ 3.4: 1	<0.05

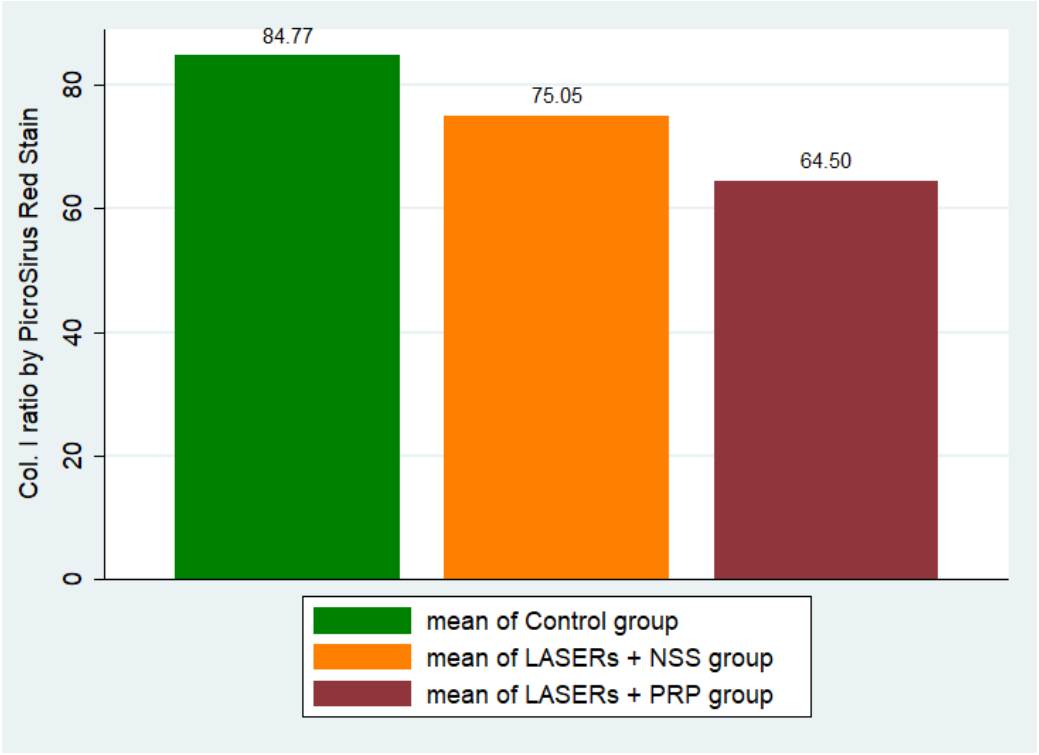
We consistently observed a significant rise in COL III (new immature COL) among control, Lasers + NSS and, Lasers + PRP groups (13.9 ± 15.97 vs 24.17 ± 21.67 vs 34.41 ± 23.57, respectively, repeated-measures ANOVA, F(2,60)=38.66, P<0.0001) as shown in figure 30.

Figure 30: COL II ratios across groups



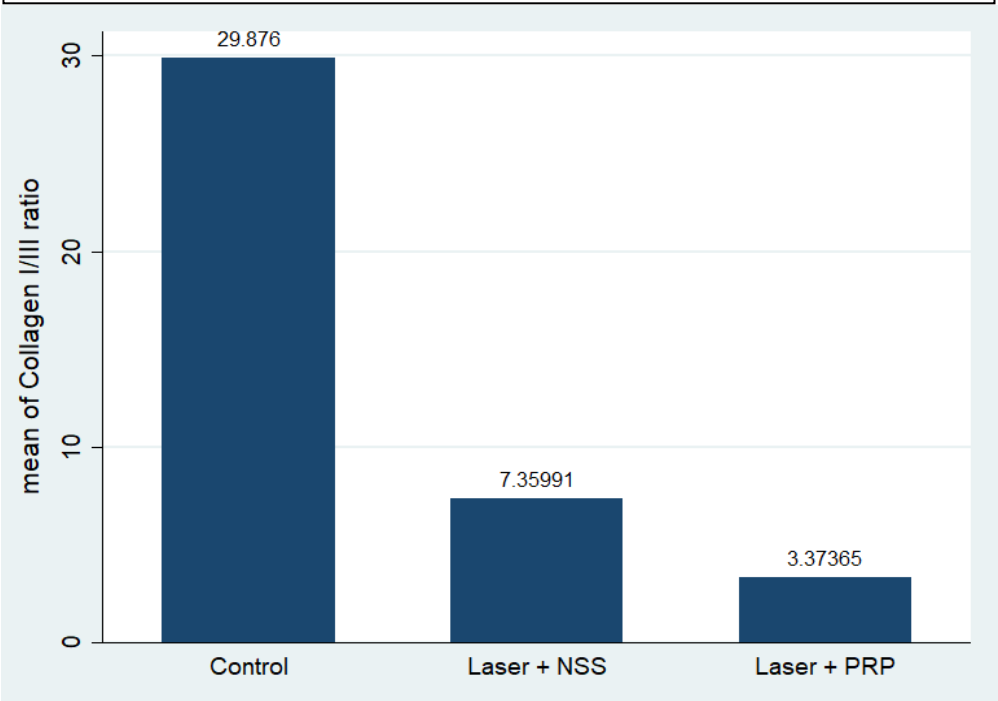
On the other hand, we observed a simultaneous significant decrease in COL III (old mature COL) among control, Lasers + NSS and, Lasers + PRP groups (84.77 ± 15.6 vs 75.05 ± 21.05 vs 64.5 ± 23.13, respectively, repeated-measures ANOVA, F(2,60)=38.80, P<0.0001) as shown in figure 31.

Figure 31: COL I ratios across groups



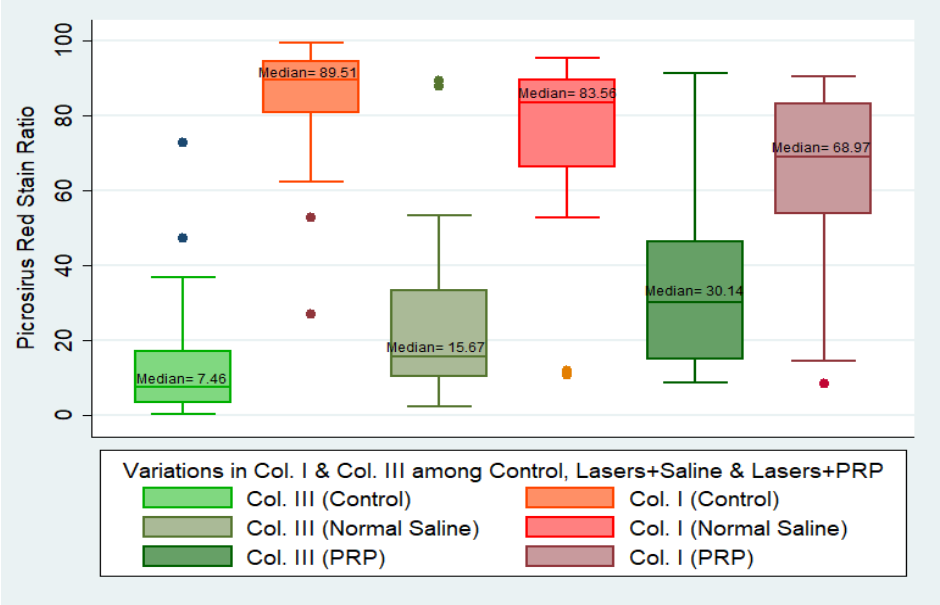
Similarly, COL I/III has shown significant decrease among control, Lasers + NSS and, Lasers + PRP groups (≃ 29.9: 1 vs ≃ 7.4: 1 vs ≃ 3.4: 1, respectively, repeated-measures ANOVA, $F(2,60)=5.78$, $P<0.05$) as shown in figure 32.

Figure 32: mean COL I/III ratios among groups



The medians of COL III and COL I for control, Laser plus normal saline and Laser plus PRP are shown in the box and whisker graph (Figure 33).

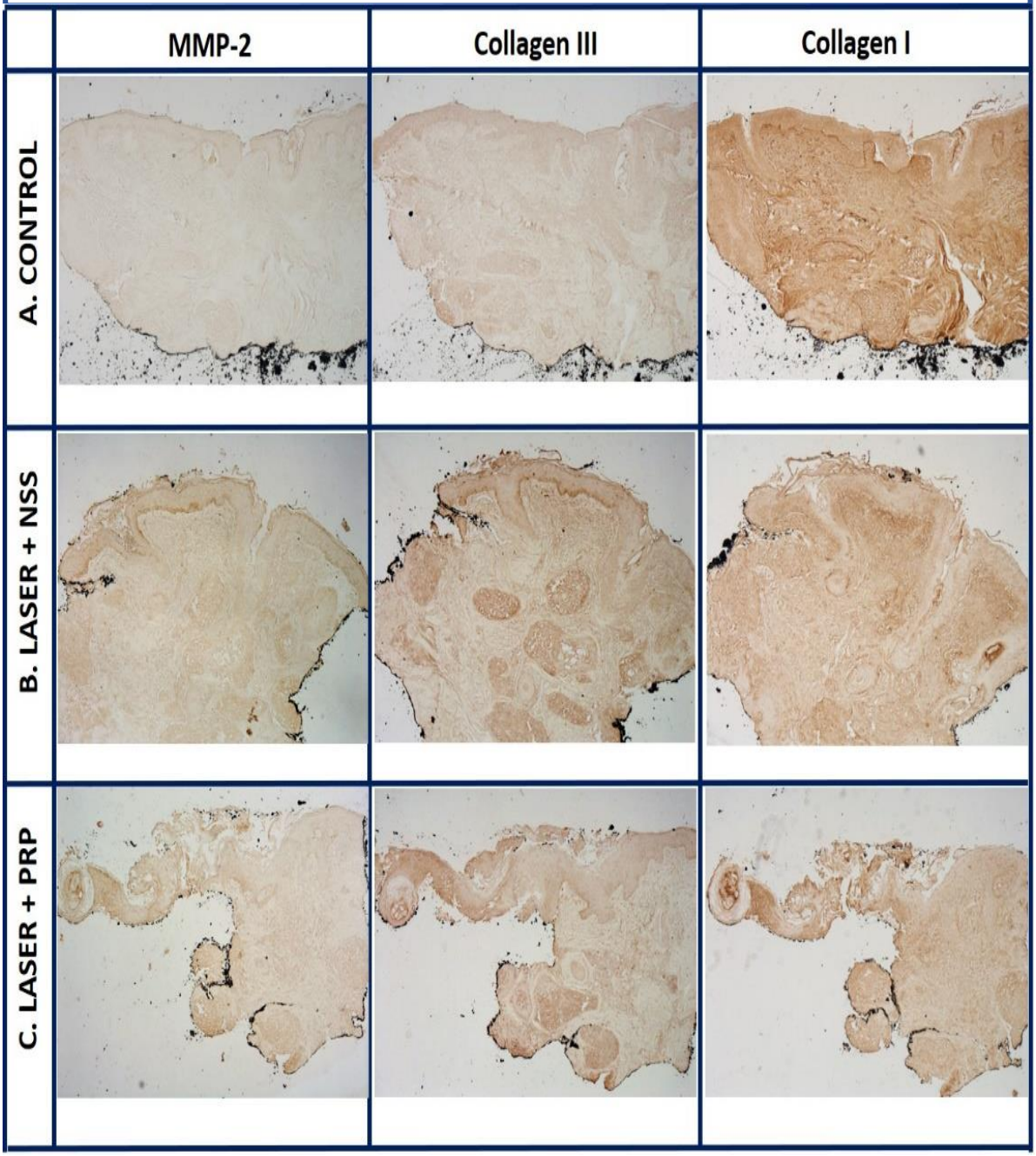
Figure 33: mean COL I/III ratios among groups



6.3.3. Immunohistochemical staining of COL III, I and MMP-2

For further evaluation, immunohistochemical staining was performed for all biopsies.

Figure 34. Biopsies of Case no. 8 showing Immunohistochemical staining for MMP-2, Col. III, and Col. I for a) Control, b) LASER + NSS, and c) LASER + PRP



The following table 17 shows the immunohistochemical results for COL I, COL III and MMP-2 among the 3 groups.

Table 17: IHC ordinal categories for Col. I, Col. III and MMP-2 among groups

Serial	Control			Laser + NSS			Laser + PRP		
	Col. I	Col. III	MMP-2	Col. I	Col. III	MMP-2	Col. I	Col. III	MMP-2
1	4	2	0	3	2	3	3	2	2
2	3	2	3	3	2	2	3	2	2
3	3	2	0	4	2	0	3	3	0
4	4	2	3	4	2	4	3	2	4
5	4	2	0	3	1	0	3	1	0
6	3	2	3	3	2	0	3	2	0
7	2	2	0	2	3	2	2	2	2
8	3	0	0	3	1	0	2	2	0
9									
10	3	1	4	3	2	2	2	2	4
11	3	3	3	3	2	2	3	2	0
12	4	2	3	4	3	4	4	3	0
13	4	2	2	2	2	2	3	1	2
14	2	2	3	3	2	0	3	2	2
15	3	2	0	2	2	0	3	1	0
16	3	2	0	4	2	0	3	2	1
17	3	2	4	4	2	0	4	2	2
18	3	2	0	3	2	3	3	2	3
19	3	0	0	3	0	0	2	2	0
20	2	2	2	2	2	3	3	2	4
21	2	3	0	3	2	0	2	1	2
22	3	1	0	3	1	0	3	1	0
23	3	1	0	3	1	0	3	2	0
24	3	1	2	3	2	2	3	2	0
25	3	1	2	4	2	2	3	1	2
26	3	2	2	4	2	2	3	2	2
27	3	2	2	3	2	0	3	2	2
28	3	2	2	2	2	0	3	3	2
29	3	2	2	4	2	2	3	2	3
30	3	2	0	3	2	0	4	2	2
31	4	2	0	4	2	0	3	2	3
32	2	2	0	3	1	0	3	1	0

Unlike Picrosirius Red Stain, COL III showed no significant difference among the 3 groups (Friedman's test =37.53, p= 0.16). Similarly, COL I showed no significant difference among the 3 groups (Friedman's test =39.54, p= 0.11). However, MMP-2 has

shown a significant difference among the 3 groups (Friedman = 46.99, p= 0.04). The descriptive statistics for MMP-2 in the 3 groups are shown in the following table 18.

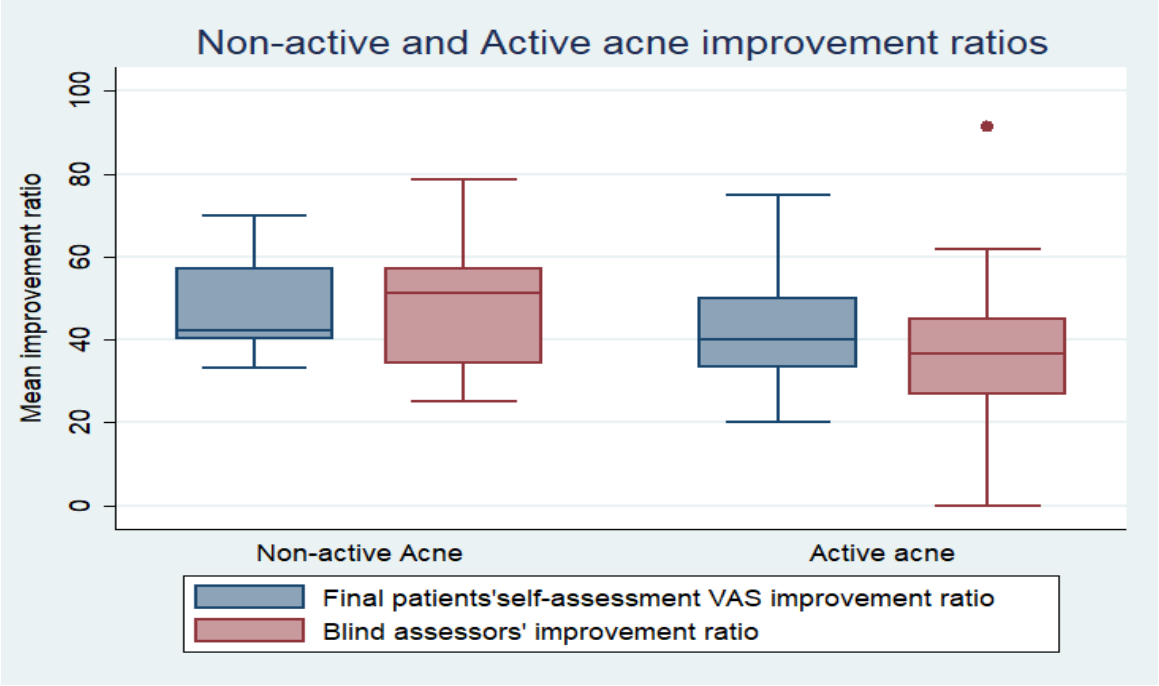
Table 18: MMP-2 in Control, Laser + NSS and Laser + PRP groups

Group	Mean ± SD	Q1	Median	Q3
Control	1.35 ± 1.40	0	2	2
Lasers + NSS	1.03 ± 1.27	0	0	2
Lasers + PRP	1.61 ± 1.35	0	2	2

6.4. Active Acne effect on improvement

Although improvement of non-active acne group was higher than active acne group, there were no significant differences neither by final patients' self-assessment VAS score nor blind assessors' mean improvement ratio between the two groups (Figure 35). Final patients' self-assessment showed no significant difference between non-active and active acne groups (47.46±11.24 vs 41.75±11.99, respectively, p= 0.15, Mann-Whitney Rank Sum Test). Similarly, mean blind assessors' scores showed no significant difference between non-active and active acne groups (47.47±15.38 vs 37.05±19.31, respectively p= 0.1, Mann-Whitney Rank Sum Test).

Figure 35 : mean improvement by patients and blind assessors for active and non-active acne



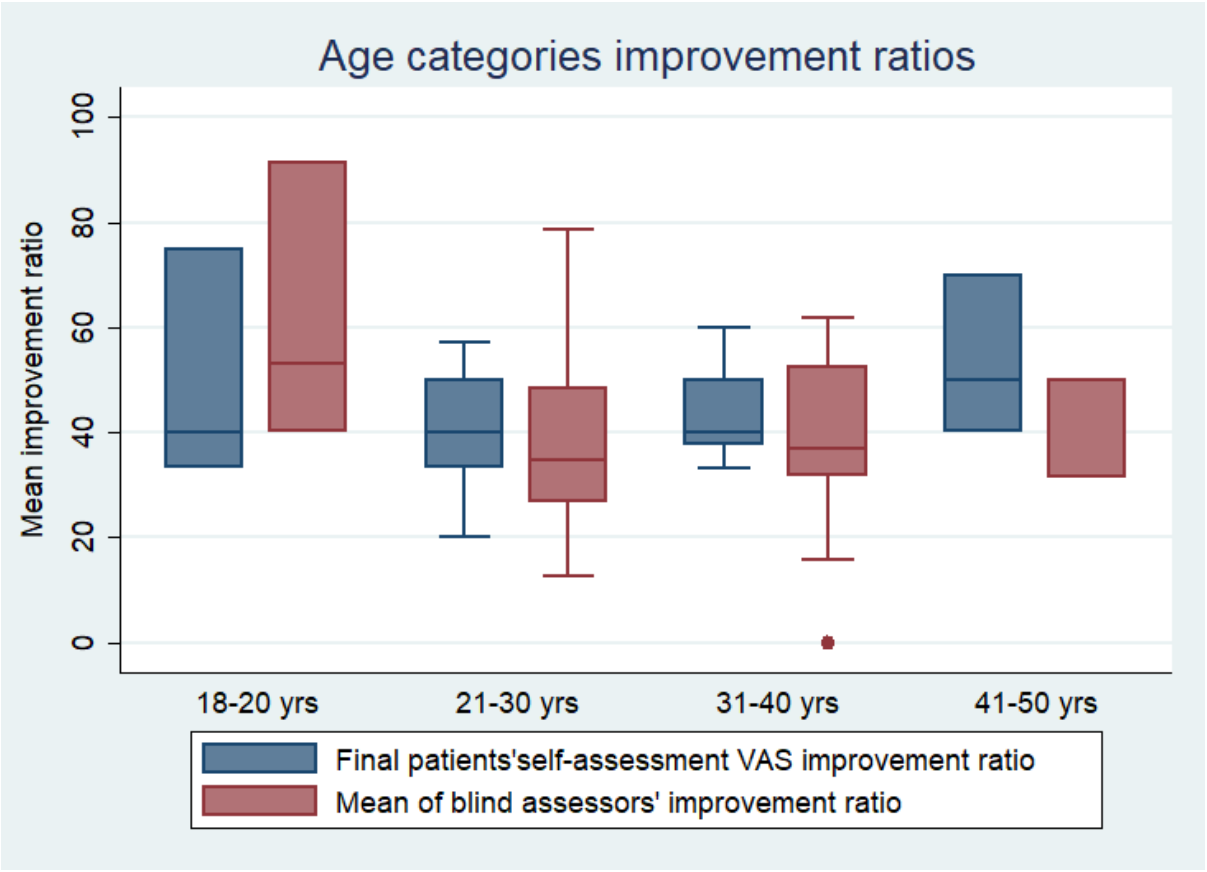
6.5. Age category effect on improvement

The mean±SD among different age categories is shown in the following table 18. Although the oldest age group (41-50 yrs) showed the highest improvement ratio (53.33%) by final patients' self-assessment VAS score, the youngest age group (18-20 yrs) showed the highest improvement ratio (61.42%) by mean blind assessors (Figure 36). However, there were no significant differences among the four age category groups neither by final patients' self-assessment (ANOVA, p=0.35) nor blind assessors (ANOVA, p=0.23).

Table 18: Final improvement among age categories

Age Category	Final patients' self-assessment VAS score (mean ±SD)	Mean blind assessors' score (mean ±SD)
18-20 yrs	49.44±22.38	61.42±26.68
21-30 yrs	40.59±12.90	39.22±18.64
31-40 yrs	43.25±7.44	37.72±16.6
41-50 yrs	53.33±15.28	43.75±10.83

Figure 36: Improvement by patients and blind assessors among age categories



7. Discussion

Discussion

The early clinical improvement through a patient self-assessment after the first session was a primary outcome in our study as a motivation for participants to continue the course of treatment. In our study, one patient (3%) had clinical improvement of 76% to 100%, eight (25.8%) had improvements of 51% to 75%, eleven (35.48%) had moderate improvements of 26% to 50%, and eleven (35.48%) had minimal to no improvement, based on patients' self-assessment after the first session. The mean grade of clinical improvement based on patients' self-assessment was 2.42 ± 1.78.

Cho and his colleagues have published two studies for evaluations of a single fractional lasers session in post-acne scars. In their first study, twenty Korean patients with atrophic acne scars were treated with a single session of two modes of CO_2 AFL. The laser fluences were delivered to the scars using the Deep FX mode and additional treatment using the Active FX mode was performed throughout the entire face. The patient surveys regarding overall satisfaction revealed that 12 of the 20 patients (60.0%) were very satisfied or satisfied, five (25.0%) were slightly satisfied, and three (15.0%) were unsatisfied. The mean grade of clinical improvement, based on the dermatological clinical assessment, was 2.4.(129)

In their second study, they compared the efficacy and safety of single-session treatment of Erbium 1,550 nm NAFL and CO_2 AFL for acne scars through a randomized, split-face, evaluator-blinded study. Eight patients with acne scars were enrolled in this study. Half of each subject's face was treated with NAFL and the other half was treated with CO_2 AFL. For the Erbium NAFL, no patient had clinical improvement of (0%) 76% to 100%, two (25.0%) had improvements of 51% to 75%, five (62.5%) had moderate improvements of 26% to 50%, and one (12.5%) had minimal to no improvement. While for the CO_2 AFL, two (25.0%) patients had clinical improvement of 76% to 100%, four (50.0%) had improvements of 51% to 75%, one (12.5%) had moderate improvement of 26% to 50%, and one (12.5%) had minimal to no improvement. The mean grade of clinical improvement based on clinical assessment was 2.0 ± 0.5 for NAFL and 2.5 ± 0.8 for AFL.(46)

The two previously discussed studies evaluated patients 3 months after a single session, while in our study we considered an earlier evaluation only after 1 month. This difference in the evaluation timeline and larger cohort size in our study (32 patients versus 20 and 8 patients) may justify the variation from our results.

Few studies have compared the results of fractional ablative versus non-ablative lasers in post-acne scars.(122, 130, 147-149) A significantly higher results were reported

with the fractional ablative lasers with a higher incidence of adverse effects. However, Kim and his colleagues has conducted a single split-face study in 2009 comparing the combination of AFL (10,600 nm CO_2) and NAFL (1,064-nm Nd: YAG laser) on one half to dual AFL (10,600 nm CO_2) using high (Group A) and low energy (Group B) on the other half. A series of 20 patients (skin phototypes IV–V) with atrophic facial acne scars received three successive monthly treatments. Patients were evaluated using digital photography at each treatment visit and at 3 months postoperatively. Clinical assessment scores were determined at each treatment session and follow-up visit, but no histopathological assessment was performed. They concluded that the combination of AFL resurfacing and NAFL resurfacing yielded the best results, as assessed in photographs as well as by the overall appearance of the acne scars with fewer complications. Similarly, we agreed on the lack of need for the CO_2 AFL with high energy and intensity on areas that include normal skin. Comparing results, we reported that 32.25% of our patients achieved > 50% improvement, while in their best group of low energy AFL + NAFL they reported that 100% of patients achieved > 50% improvement.(45) There could be many reasons for such a difference including; their use of AFL for the whole face, the use of NAFL 1,064 nm Nd:YAG laser which could reach deeper than NAFL 1,550 nm Erbium laser, and the assessment method (we had 4 blind assessors, while they have 2 non-blind assessors).

One year later, a similar letter published on a single post-acne scars patient reported the effects of combined use of 1,550 nm Erbium-doped NAFL and 10,600 nm CO2 AFL. They suggested that such a combination can potentially improve clinical outcomes, although they observed the occurrence of post-therapy erythema and hyperpigmentation, which persisted over several months. According to the authors, these unexpected outcomes may have resulted from bulk heat damage to the surrounding tissues by heat stacking.(140) Unlike our study they applied four passes with the NAFL Fraxel SR1500 (settings of 40 J/cm2 and density 17% coverage) followed by treatment with AFL Ultrapulse Encore laser in the active FX™ mode (settings of 100 mJ and 55% coverage/cm2 per pass, one pass without overlapping); both NAFL and AFL for the whole face. Such a difference in the application on the whole face, the sequence of LASERs and the absence of PRP could be the reason for the lower downtime in our study.

In our control group of atrophic post-acne scars, COL III and COL I expression values were (13.9 ± 15.97) and (84.77 ± 15.6), respectively. The two dominant types of

COL in wound repair are COL I and III. In normal skin, COL fibrils are composed of both COL I and III with COL III comprising 20% of the total.(150) Both COL types are responsible for maintaining skin integrity, while COL III provides tensile strength, flexibility and softness of skin.(151) The high variation in COL III expression we observed could be due to different nature of atrophic scars in our control group, the inclusion of surrounding skin treated by NAFL, and the predominant location of COL III in reticular dermis, while COL I is mostly located in the deep dermis.

An old study suggested that during the early stages of granulation tissue formation, myofibroblasts lay down COL III. COL III expression increases more than the COL I expression in the early stages of healing, resulting in increased ratio between the two COL subtypes from 20% up to 50% COL III.(152) The results in our study confirmed similar findings, where the means were (24.17$\pm$21.67) for COL III and (75.05$\pm$21.05) for COL I in Lasers plus NSS group, while, the means were (34.41$\pm$23.57) for COL III and (64.5$\pm$23.13) for COL I in Lasers plus PRP group. Such a rise in COL III proved that lasers effect on scars remodelling started very early. Amazingly, there was a significant difference between Lasers plus NSS and Lasers plus PRP groups for both COL types ($p<0.0001$). Moreover, repeated-measures ANOVA showed significant differences for both COL III and COL I ($p<0.0001$).

Another study using an objective histopathological analysis for differences in COL architecture among different scar types and normal skin has concluded that COL I/III ratios were 5:1 for unwounded skin, 6:1 for normal scars, 6:1 for hypertrophic scars, and 17:1 for keloids.(153) Those results were inconsistent with our results, where the mean of COL I/III ratio was 30.1: 1 for control group of post-acne scars. The reason for such a variation may be the computerized ImageJ higher accuracy of detection for COL I and COL III in our study. Unlike the control group, the means of COL I/III ratio for Lasers plus NSS and Lasers plus PRP groups were 7.7: 1 and 3.4:1, respectively. ANOVA among the three groups showed high significance for COL I/III ratio ($p=0.0056$). These findings were consistent with an earlier study conducted on burns to illustrate increase in type III COL expression and decrease in type I COL expression after 3 sessions of CO_2 AFL.(154) However, scars in our study were biopsied immediately after the first session.

In our study, the intensity of immunohistochemical staining for COL III and I was not significantly different among the three groups. This was inconsistent with results for immunohistochemical investigation of wound healing in response to CO_2 AFL, in which

proCOL III was early detected in biopsies.(155) This inconsistency may be due to the difference in our technique in fixation and late unmasking of the epitopes. On the other hand, MMP-2 immunohistochemical evaluation has shown a statistically significant difference among the three groups (p=0.029). Normally, the expression of MMPs in intact skin is very low. However, after skin injury, multiple MMPs are produced during the normal healing process, where MMP-2 is secreted early by fibroblasts remains stable throughout healing.(156) Thus, the initiation of early healing process was confirmed in our findings.

The pursuit of good results in treating post-acne scars with fractional ablative CO_2 and non-ablative Erbium: Glass lasers has been widely studied.(46, 90-94, 130, 143) Kim and colleagues were the first to report the fractional Erbium 1,550 nm Laser in post-acne scars patients.(89) However, since the fractional photothermolysis concept has been applied to the CO_2 laser, this modality has virtually replaced traditional CO_2 laser resurfacing. Thus, it was possible to treat post-acne scars without a high incidence of dyschromia and/or hypertrophic scarring.

In the fractional modality, an array of microthermal zones (MTZ) is created within the epidermis and dermis. These MTZs consist of a central ablation area surrounded by a thin annular area of coagulated tissue. In between the MTZs, there is healthy tissue, which is said to be responsible for the faster recovery time. This MTZ column pattern allows fractional CO_2 laser to reach deeper areas into the dermis than traditional CO_2 laser. Later, another option of increasing depth was also possible by means of overlapping pulses at the same site (stacking). Oni and his colleagues demonstrated that a 15mJ double pulse resulted in similar MTZ as a single 30mJ pulse. They suggested that double pulsing at lower energies may result in better clinical outcomes than increasing energies or using multiple passes at single pulse, as the amount of unaffected tissue between MTZs was reduced.(157) This would increase dermal injury leading to increased COL synthesis, while minimizing the spread of surrounding coagulation zone around MTZs.

An earlier study was performed by a pinpoint irradiation technique using only the standard CO_2 laser without the fractional laser device in the treatment of atrophic acne scars. Thirty-five patients were treated up to five sessions at 2–3-week intervals. The authors achieved a noticeable clinical improvement with minimal side effects. Moreover, the subsequent use of PRP injections following fractional ablative CO_2 laser which was first reported by Lee and his colleagues showed an enhanced recovery of laser-damaged

skin and synergistic improvement for the clinical appearance of acne scarring after one session.(56)

Based on those studies, we conducted our study using a five-stack fractional ablative CO_2 laser on the post-acne scars followed by PRP injections. However, in our study, we proposed a different technique by limiting the high power deep fractional CO_2 laser irradiation only to the post-acne scars followed by intracicatricial PRP injections into the core of the scars. For further enhancement of the results, we combined the 1,550 nm NAFL Erbium for the whole face as a second step after spot fractional CO_2 laser before PRP injections. Thus, the aim of our study was to maximize the effect of this combination on post-acne scars to achieve the best results.

To our knowledge, this is the first study to investigate a triple treatment by AFL, NAFL and PRP, all in one session for treatment of atrophic post-acne scars. Variability in types and depth of atrophic post-acne scars was the impulse for proposing such a new combination. The early improvement in FASQoL after the first session for patients denoted the efficacy of this combination with minimal side effects.

-

8. Conclusions

Conclusions

1. Combinations of AFL, NAFL, and PRP for treatment of post-acne atrophic scars yield earlier significant improvements when used simultaneously from the first session. This triple combination enhanced the scar remodeling with minimal adverse effects based on patients´ self-assessment 4 weeks after the first session.

2. The significant final clinical improvement of post-acne scars based on the patients' self-assessment and blind assessors' evaluation proved that lowering the parameters of AFL CO_2 during the consecutive sessions to be less ablative could be followed as a protocol for balancing improvement with possible adverse effects of laser combinations.

3. Intradermal injection of PRP into scars proved to be an effective addition to lasers combination in enhancing neocollagenogenesis as concluded from comparing biopsies of Lasers plus NSS to Lasers plus PRP.

4. Circularly Polarized Microscopy for PSR Stained biopsies proved to be a good tool for the quantitation of COL types I and III. Semi-Automated color analysis by ImageJ program for the digital photographs captured from the microscope is another powerful objective tool when applied after exclusion of background.

5. Immunohistochemical staining for COL I and III in paraffin-embedded sections may not be helpful if biopsies are kept for a long time in 4% buffered formalin due to potential loss of epitopes.

9. FUTURE LINES OF RESEARCH

Future Lines

1. A future study will focus on comparing variation of AFL parameters through consecutive sessions and measuring the effects of such variations on clinical improvement.

2. Another line of research will be a split-face comparison between combined AFL and NAFL only to combined AFL, NAFL, and PRP. This would further prove the value of PRP in improving results and minimizing adverse effects including downtime for recovery.

3. The effect of combined lasers on different subtypes of post-acne scars with histopathological assessment will be another future topic of research.

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11. Annexes

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INFORMATION SHEET FOR PARTICIPANTS

Study title: "Combined Fractional LASERs Resurfacing with Platelets Rich Plasma (PRP) for treating Post-acne atrophic scarring"

Study code: ClinicalTrials.gov ID: NCT03809416

Promotor: Dr. Ahmed Abdelsalam Youssef Ibrahim. LASER Unit, Cosmetic Surgery Clinic (CSC), Kuwait

Introduction:

We invite you to participate in a clinical trial of a combined treatment for post acne atrophic scars. You are free to decide whether you want to participate in this study. Before deciding, please read the following information and discuss it with the researcher (the doctor responsible for the study).

This study has been approved by the corresponding Ethics Committee of Cosmetic Surgery Clinic-Kuwait.

Our intention is only that you receive the correct and sufficient information so that you can evaluate and judge whether you want to participate in this study. To do this, read this fact sheet carefully and we will clarify any doubts that may arise after the explanation. In addition, you can consult with the people you deem appropriate.

Voluntary Participation

You should know that participation in this study is voluntary and that you can decide not to participate or change your decision and withdraw your consent at any time, without thereby altering the relationship with your doctor or causing any harm in your treatment.

General description of the study

Acne has a prevalence of over 90% among adolescents and persists into adulthood in approximately 12%–14% of cases with psychological and social implications of high gravity. Atrophic acne scarring is a permanent complication of acne vulgaris, which may be associated

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with significant psychological distress. All body areas with high concentrations of pilosebaceous glands are involved, particularly the face, back and chest. Inflammatory acne lesions can result in permanent scars, the severity of which may depend on delays in treating acne patients. General dermatologists are frequently presented with the challenge of evaluating and providing treatment to patients with acne scarring. The incidence of acne scarring is not well studied, but it may occur to some degree in 95% of patients with acne vulgaris. Studies for general population reported incidence of acne scarring ranging from 1 to 11 percent. The importance of acne scarring treatment came from linkage to poor self-esteem, depression, anxiety, altered social interactions, body image alterations, embarrassment, anger, lower academic performance and unemployment.

Our experience with lasers treatment is that it is the most useful as and, in our impression, that if combined lasers are used, better results with less side effects could be achieved but this has been demonstrated in few studies with little evidence.

In this study, we propose a new combined technique using two different laser wavelengths and Platelets Rich Plasma (PRP) to treat post atrophic acne scars. Within the available knowledge of laser tissue interactions and effects of PRP on wound healing, we will explore the clinical effects of our new combination procedure on histopathological basis for guiding future post acne scars clinical research.

To verify that such a combination is useful in the treatment of acne scars, we will need to compare it with a control, where a biopsy taken by small punch excision will be taken during the 1st session. Two more small punch excision biopsies will be taken from another two scars after treatment with lasers plus normal saline solution and lasers plus PRP during the 1st session, also. These biopsies will be taken from the worst three scars after agreement with you. The three linear minute residual scars will be treated by lasers in the three subsequent sessions.

Benefits and risks derived from participation in the study

Platelet-rich plasma (PRP) is a high concentration of platelets in a small volume of plasma. PRP contains various growth factors and cytokines released by platelets, and those substances play a critical role in all aspects of the wound healing process. The wound healing effect of PRP is relatively well known, and PRP has been used in bone surgery, tendon and ligament repair, and chronic leg ulcer treatment. No side effects has been reported for autologous PRP use.

Fractional photothermolysis was described as a new method for delivery of laser energy with the potential of laser safety and efficacy. Through delivery of microscopic, non-contagious zones of thermal damage using a 1550 nm, mid infra-red laser source, it was

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observed that surrounding islands of dermal and epidermal cells facilitated post treatment collagen remodeling and rapid healing. Despite the success of minimally ablative and fractional technologies, there remained a need for more aggressive tissue ablation for the purposes of tissue rejuvenation. This might be due to the variability of architecture, depth and width; thus, each type of scar has an optimal method by which it can be improved. The idea of combining both carbon dioxide and erbium lasers appeared even before the era of fractional lasers.

The combination of fractional lasers, carbon dioxide and erbium-doped laser, for treating mild acne scars has been investigated once before. The investigator reported a longer post laser erythema and hyperpigmentation, without a precise pathogenesis. However, they suggested that these unexpected outcomes may have resulted from bulk heat damage to the surrounding tissues by heat stacking and recommended further studies to determine the optimal treatment parameters and reduce unexpected adverse reactions

These results, together with our clinical experience, indicate that lasers and PRP could be safe for use in acne scars. Also, in this study we will monitor your response after 1st session through two new patient-oriented tools, to make sure you have benefit from the treatment.

It cannot be assured that if you participate in the study, you will benefit from the combination treatment. However, your participation in this study will allow researchers to better understand how this new combination could help improve the treatment of atrophic acne scars, thus benefiting other patients who also suffer from this disease.

Like any lasers, the combination being investigated can produce more or less side effects in some patients. Considering the protocol for this study and the experience of using lasers, it would be reasonable to assume that there will be no undesirable effects other than those already explained.

Do not hesitate to ask your doctor for advice if any of these symptoms occur or if you believe that you cannot tolerate the study treatment. If necessary, the investigator may choose to discontinue treatment and withdraw you from the study.

You will be asked not to take any other medication, including over-the-counter medications, without your doctor's consent. In all cases, do not forget to inform the doctor in charge of the investigation of any unusual symptoms, whether it seems to be related to your participation in the study or not.

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Alternative treatments

The alternative treatment to lasers plus PRP treatment in your case include chemical peelings, dermabrasion, Subcision, fillers, stem cell, fat grafting, punch excision and elevation.
In case there is no improvement, you will be given the option of shifting to another treatment modality, which could be useful for your scars. If it is necessary to start another treatment modality, you will no longer participate in the study, but will be continuously monitored with the new treatment option.
The doctor in charge of this study can provide you with all the necessary information in case you need it.

Patient protection

This study has been organized in accordance with the Declaration of Helsinki, the guidelines of Good Clinical Practice and national regulations.

The study protocol has been reviewed by an Ethics Committee; whose task is to verify that the necessary requirements for the protection of your rights have been met. The Ethics Committee issued a favourable opinion before the start of the study.

Confidentiality

The processing, communication and transfer of personal data of all participating subjects will be in accordance with the provisions of Organic Law 15/1999, of December 13 on the protection of personal data. In accordance with the provisions of the aforementioned legislation, you can exercise the rights of access, modification, opposition and cancellation of data, for which you should contact the study doctor.
The data collected for the study will be identified by a code and only the study doctor / collaborators will be able to relate said data with your medical history. Therefore, your identity will not be disclosed to any person except for exceptions, for example, ethics committee or in case of medical urgency or legal requirement.
Access to your personal information will be restricted to the study doctor / collaborators, health authorities, the Clinical Research Ethics Committee and personnel authorized by the promoter, when they need to check the data and procedures of the study, but always maintaining the confidentiality of them according to current legislation.

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Decision to participate in the study

You are free to decide whether you participate in this study. You can refuse and even if you accept, you can withdraw yourself from the study at any time without justifying your decision and without incurring any responsibility.

Your relationship with the medical team will not be affected at all by your decision. The doctor will continue to treat you with the available alternative treatments.

If new information appears during the study that could affect your decision to participate in the study, you and the study doctor will be informed and asked to sign a new consent. Your refusal or withdrawal from the study will have no effect on management of your case in the future and the doctor will continue to treat you with the available alternative treatments.

If you decide to remove yourself from the study, please inform the doctor responsible for your decision so that you can take appropriate safety measures if necessary. After your withdrawal, your data will be no more collected for the study.

The doctor responsible for the study could decide at any time to end the study without consent, if he thinks there is a reason to make this decision.

If you accept to participate in this study, you cannot participate in another study simultaneously and within 3 months of the end of the present study.

Organization and financing of research

This is a study promoted by the researcher without any sponsorship. The doctor and collaborators of this study will not receive any kind of financial compensation.

Economic compensation

Your participation in the study will incur free PRP for all the sessions. You will not receive financial compensation for your participation in this clinical trial.

Study results

You have the right to be informed of the overall results of this study. When available, you can request them from the research doctor.

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INFORMED CONSENT

Study title: "Combined Fractional LASERs Resurfacing with Platelets Rich Plasma (PRP) for treating Post-acne atrophic scarring"

Study code: ClinicalTrials.gov ID: NCT03809416

I (name and surname) _____

I have read the information sheet given to me. I have been able to ask questions about the study.
I have received enough information about the study. I have spoken with:
(name and surname of the researcher)
I understand that my participation is voluntary.
I understand that I can withdraw from the study:
1st whenever I want
2nd without having to explain.
3rd without this having an impact on my medical care.

- I freely give my consent for my participation in the study and give my consent for the access and use of my data, as well as the taking of photographs in the conditions detailed in the information sheet.

_____ _____
Name, Surname and Signature of the participant **Date**

_____ _____
Name, Surname and Signature of the Person who obtains the Consent Date **Date**

This document will be signed in duplicate with one copy remaining for the investigator and another for the patient.

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ClinicalTrials.gov PRS
Protocol Registration and Results System

ClinicalTrials.gov PRS DRAFT Receipt (Working Version)
Last Update: 11/26/2019 15:43

ClinicalTrials.gov ID: NCT03809416

Study Identification

Unique Protocol ID:	AY001
Brief Title:	Combined LASERs and PRP for Postacne Scars
Official Title:	Combined Fractional LASERs Resurfacing With Platelets Rich Plasma (PRP) for Treating Post Acne Atrophic Scarring
Secondary IDs:	

Study Status

Record Verification:	November 2019
Overall Status:	Completed
Study Start:	October 1, 2018 [Actual]
Primary Completion:	June 30, 2019 [Actual]
Study Completion:	October 30, 2019 [Actual]

Sponsor/Collaborators

Sponsor:	DEKA S.r.l.
Responsible Party:	Sponsor
Collaborators:	Universitat Autonoma de Barcelona

Oversight

U.S. FDA-regulated Drug:	No
U.S. FDA-regulated Device:	No
Unapproved/Uncleared Device:	No
U.S. FDA IND/IDE:	No
Human Subjects Review:	Board Status: Approved Approval Number: 2018AY001 Board Name: CSC Board Affiliation: Cosmetic Surgery Clinic - Kuwait Phone: 0096522390086 Email: drahmedyderma@gmail.com Address: P.O. Box 16789, Qadisiyah 35858, Kuwait
Data Monitoring:	Yes

FDA Regulated Intervention: No

Study Description

Brief Summary: This study evaluates a new combined technique using two different laser wavelengths and Platelets Rich Plasma (PRP) to treat post-atrophic acne scars. Within the available knowledge of laser-tissue interactions and effects of PRP on wound healing, we will explore the clinical effects of our new combination procedure on a histopathological and immunohistochemical basis for guiding future post acne scars clinical research.

Detailed Description: The carbon dioxide and erbium lasers have been the gold and silver standards for acne scars treatment. As with selective photothermolysis, a major advance in the field is the incorporation of grids of MicroThermal Zones (MTZ) that spares islands of skin with an attractive treatment efficacy to downtime healing (5-7 days) ratio. Application of these fractionated resurfacing to carbon dioxide and erbium lasers allows deeper penetration into the skin.

Fractional photothermolysis was first described by as a new method for delivery of laser energy with the potential of laser safety and efficacy. Through the delivery of microscopic, non-contagious zones of thermal damage using a 1550 nm, mid-infra-red laser source, it was observed that surrounding islands of dermal and epidermal cells facilitated post-treatment collagen remodeling and rapid healing. Despite the success of minimally ablative and fractional technologies, there remained a need for more aggressive tissue ablation for the purposes of tissue rejuvenation of severely photodamaged skin and deeper rhytides. This might be due to the variability of architecture, depth, and width; thus, each type of scar has an optimal method by which it can be improved.

The idea of combining both carbon dioxide and erbium lasers appeared even before the era of fractional lasers. McDaniel et al, combined both non-fractional lasers for resurfacing of perioral rhytides comparing it to using carbon dioxide lasers alone and concluded that carbon dioxide laser resurfacing followed by 3 passes of erbium laser reduces the duration of crusting, swelling and itching when compared to carbon dioxide laser resurfacing alone with no significant difference in the outcome.

Later in 2010, the combination of fractional lasers carbon dioxide and erbium-doped laser was tried out for treating mild acne scars by a group of researchers. They reported a longer post laser erythema and hyperpigmentation, without precise pathogenesis. However, they suggested that these unexpected outcomes may have resulted from bulk heat damage to the surrounding tissues by heat stacking and recommended further studies to determine the optimal treatment parameters and reduce unexpected adverse reactions.

Platelet-rich plasma (PRP) is a high concentration of platelets in a small volume of plasma. PRP contains various growth factors and cytokines released by platelets, and those substances play a critical role in all aspects of the wound healing process. Among the stored mitogenic factors essential for wound repair are platelet-derived growth factor (PDGF) with the -AB and -C isoforms predominating, transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived epidermal growth factor (PDEGF) and insulin-like growth factor-1 (IGF-1). These are variously involved in stimulating chemotaxis, cell proliferation, and maturation. PDGF is a powerful chemoattractant and stimulator of cell proliferation. All of them are potent angiogenic factors and endothelial cell mitogens. The wound healing effect of PRP is relatively well known, and PRP has been used in bone surgery, tendon and ligament repair, and chronic leg ulcer treatment.

Conditions

Conditions: Atrophic Acne Scar

Keywords: Acne scars
fractional lasers
PRP

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: N/A

Interventional Study Model: Factorial Assignment

Number of Arms: 3

Masking: Double (Investigator, Outcomes Assessor)

Allocation: Non-Randomized

Enrollment: 32 [Actual]

Arms and Interventions

Arms	Assigned Interventions
No Intervention: Control 32 biopsy specimens from acne scars will be excised without any treatment.	
Sham Comparator: Lasers plus Normal Saline Solution 32 biopsy specimens will be excised immediately after being treated with lasers and subsequent injection of normal saline solution.	Device: Lasers Fractional carbon dioxide laser will be applied only over spots of deep post-acne scars, while Erbium: Glass will be applied all over face except to the two predetermined spots, i.e. control and another spot where NSS will be injected. Other Names: • Fractional CO2 and Erbium Glass Lasers Combination Product: Lasers plus Normal Saline Solution Normal Saline Solution will be injected only in one spot after combined lasers resufacing to act as sham comparator compared to the other spot of Lasers plus PRP injection Other Names: • NSS
Active Comparator: Lasers plus Platelets Rich Plasma (PRP) 32 biopsy specimens will be excised immediately after being treated with lasers and subsequent injection of platelets Rich Plasma (PRP).	Device: Lasers Fractional carbon dioxide laser will be applied only over spots of deep post-acne scars, while Erbium: Glass will be applied all over face except to the two predetermined spots, i.e. control and another spot where NSS will be injected. Other Names: • Fractional CO2 and Erbium Glass Lasers Platelets Rich Plasma

Arms	Assigned Interventions
	Platelet Rich Plasma (PRP) will be prepared from autologous blood collection in a syringe prefilled with anticoagulant solution followed by centrifugation then adding calcium gluconate 10% for induction of platelet activation. Activated PRP will be injected for all carbon dioxide laser spots in each patient immediately after each laser session except for two predetermined spots, i.e. control and sham comparator, which will be injected by normal saline solution (NSS). Other Names: • PRP

Outcome Measures

Primary Outcome Measure:

1. Change in Patients' response denoting early Clinical improvement
   calculating the change in 17 questions' responses of a new Patient Oriented Tool questionnaire for assessing Atrophic Acne Scarring through two parts; a) assessment of Acne Scar Appearance and b) Acne Scar Quality of Life questionnaire. Patients will record their responses before the 1st session and on the date of 2nd session.

   [Time Frame: 4 weeks after the 1st session]

2. Final Clinical improvement assessed using (0-10) scoring scale by blind assessors
   The change in scores comparing 2 sets of digital photographs for each patient evaluated by 3 blind assessors, before and after 12 weeks of the 4th session. This blind assessment score will be compared to patients' score in their previous questionnaire.

   [Time Frame: 12 weeks after the 4th session]

Secondary Outcome Measure:

3. Higher new collagen formation in scars treated using lasers plus PRP technique
   Picrosirus Red stained specimens under circularly polarized microscopy will be segmented into two color threshold bands (Green/Yellow G/Y and Red/Orange R/O) in the color HSB space. This will be numerically evaluated using ImageJ computer program

   [Time Frame: Immediately after the 1st session]

4. Immunohistochemical evaluation of Collagen I, Collagen III, and MMP-2
   Using a 4 grade scale (0, +, ++, +++), the 288 slides (i.e. 3 slides prepared from 96 specimens obtained from 32 patients) will be arranged for comparison among control, sham comparator and, active comparator. Also, results will be correlated to previous numerical ImageJ computerized assessment and patients' clinical improvement.

   [Time Frame: Immediately after the 1st session]

Eligibility

Minimum Age:	18 Years
Maximum Age:	
Sex:	All
Gender Based:	No
Accepts Healthy Volunteers:	Yes
Criteria:	Inclusion Criteria:

1. Patients with atrophic post-acne scars.

2. Patients without surgical &/or LASER resurfacing treatment for acne scars within the last 6 months.

Exclusion Criteria:

1. Pregnancy
2. Present or past history of hypertrophic scars or keloids
3. Present or past history of photosensitivity dermatoses including Connective Tissue Diseases.
4. Present history of herpes infection
5. Present history of Anemia (HGB < 10 g/dl), Thrombocytopenia &/or Platelets dysfunction.
6. Patients receiving isotretinoin within the last 3 months, NSAIDs within 72 hours of the procedure, anticoagulants &/or systemic use of corticosteroids within 2 weeks.

Contacts/Locations

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IPDSharing

Plan to Share IPD: No

References

Citations: **[Study Results]** Layton A, Dréno B, Finlay AY, Thiboutot D, Kang S, Lozada VT, Bourdès V, Bettoli V, Petit L, Tan J. Erratum to: New Patient-Oriented Tools for Assessing Atrophic Acne Scarring. Dermatol Ther (Heidelb). 2016 Jun;6(2):235-6. doi: 10.1007/s13555-016-0107-8. PubMed 27008237

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Links:

Available IPD/Information:

The Cosmetic Surgery Clinic

Date: July the 2nd , 2018

EC Ref No. : 2018AY001

To,

Dr. Ahmed Abdelsalam Youssef Ibrahim

Dermatology and LASER Department

Address: Virginia Building, end of 4th ring road, 7th floor, Salmiya

Subject: Approval of clinical study – by the Ethics Committee

Title of the Protocol: **"Combined Fractional LASERs Resurfacing with Platelets Rich Plasma (PRP) for treating Post-acne atrophic scarring"**

Protocol Identification: 2018AY001

Sponsor: Dr. Ahmed A. Youssef

Dear Dr. Ahmed A. Youssef,

The Ethics committee of Cosmetic Surgery Clinic – Kuwait has reviewed and discussed your application to conduct the above-mentioned clinical trial in the department of Dermatology & LASER Unit with you as the Principal investigator.

The Following documents have been reviewed and approved:

Sr. No.	Name of the Document
1	Information Sheet for participants
2	Informed consent form for participants
3	Study Protocol
4	Study Flow chart
5	Lasers parameters Sheet
6	Post Laser Instructions sheet

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The following members of the Ethics committee were present at the meeting held on (July the 2nd, 2018, Cosmetic Surgery Clinic-Kuwait).

Sr. No.	Name	Role in Ethics committee	Position	Attendance (Yes /No)	Affiliation to Institute (Yes/No)
1	Dr. Mohammed Y. ElNadi	Chairman	Medical Director	**Yes**	**Yes**
2	Dr. Tarek El-Bakry	Vice Chairman	Dermatologist	**Yes**	**Yes**
3	Sondous ElMahghoub	Secretary	Dept. Secretary	**Yes**	**Yes**
4	Christine Bruno	Member	General Manager	**Yes**	**Yes**
5	Maria Kristine A.Dela tore	Member	Registered Nurse	**Yes**	**Yes**
6	Nikka Mae O. Roda	Member	Registered Nurse	**Yes**	**Yes**

We approve the trial to be conducted in the presented form. None of the Investigator and co-investigator participating in this study took part in the decision making and voting procedure for this study

The Ethics Committee expects to be informed about the progress of the study, any side effects occurring in the course of the study, any revision in the protocol, patient information/informed consent and a copy of the final report

This Ethics Committee is working in accordance to ICH-GCP, the Helsinki Declaration guidelines and other applicable regulations.

Yours Sincerely,

Ethics Committee of Cosmetic Surgery Clinic

Virginia Building, end of 4th ring road, Salmiya

Kuwait, July the 2nd 2018

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