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Fractional ablative laser therapy for the treatment of severe burn scars: A pilot study of the underlying mechanisms☆

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ABSTRACT

Ablative fractional resurfacing is clinically an efficient treatment for burn scar management. The aim of this pilot study was to investigate the poorly understood mechanisms underlying ablative fractional CO2 laser (AFL-CO2) therapy in relation to biomarkers S100 and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). S100 stains for Langerhans cells and neuronal cells, potentially representing the pruritus experienced. 11β-HSD1 catalyses the interconversion of cortisol and cortisone in cells, promoting tissue remodelling. Immunohistochemical analysis of S100 and 11β-HSD1 protein expression in the dermis and epidermis of the skin was performed on normal skin, before and after AFL-CO2 therapy. Data assessing outcome parameters was collected concurrently with the skin biopsies. 13 patients were treated with AFL-CO2 therapy. Langerhans cells decreased by 39% after 2nd treatment. Neuronal cells were overexpressed before treatment in the scar tissue by 91% but levels returned to that resembling normal skin. 11β-HSD1 expression in keratinocytes was significantly higher after laser treatment compared to before in scar tissue ($p < 0.01$). No clear correlation was found in dermal fibroblast numbers throughout the treatment course. Whilst the role of the explored mechanisms and their association with clinical outcomes cannot conclusively be stated, this pilot study demonstrates promising trends that encourages investigation into this relationship.

1. Introduction

Scarring in human skin tissue is the result of a complex wound healing process including various molecular and signalling pathways [1]. Burn wounds are prone to hypertrophic scarring due the inflammatory nature of the injury and

☆ Work should be attributed to the same location where the work was performed.

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prolonged wound healing [2]. Characteristic of hypertrophic scarring is a firm, raised, tight and irregular scar which can result in contractures limiting the range of motion of joints [3,4]. Further, scar related symptoms such as pruritus, neuropathic pain, and the psychological impact of the injury can have a detrimental effect on the overall health and quality of life of burn survivors [4].

The positive effects seen in many scar outcome measures including the clinically assessed scar qualities, as well as neuropathic pain, pruritus and burn specific quality of life, have led to the incorporation of ablative fractional CO_2 laser therapy (AFL-CO_2) into the routine burn scar treatment in many burn units [5]. Ablative fractional laser therapy applies energy through pixelated microbeams [6]. The high laser energy induces temperatures over 100 °C in the tissues, producing columns of vaporised scar tissue alternating with columns of healthy tissue which allows the collagen in the skin/scar to remodel induced by the wound healing reaction [7,8]. Glucocorticoids are commonly used as a topical or intralesionally injected treatment after AFL -CO_2 scar therapy to assist in decreasing inflammation [24,27–30].

Research investigating laser therapy and burn scars currently focuses on improving either the hypertrophic scar characteristics [5,9,10] including the firmness, raised nature and size of the scar, or the associated markers [11–14] including CD14 and TLR4. However, to our knowledge there is no data available investigating potential markers associated with the improvement of scar related symptoms such as burn scar pruritus and pain following laser resurfacing [3,5,7,15]. The underlying biochemical mechanisms, as they relate to specific patient outcomes, are not entirely understood, specifically for burn scar associated pain and itchiness. Based on previous evidence that pruritus and neuropathic pain significantly decreases after treatment with AFL-CO_2 [6] this pilot study was performed to investigate potential relevant molecular mechanisms associated with these clinical effects.

In this pilot project, two proteins were investigated before and after laser treatment: the family of S100 proteins and the 11β-HSD type 1 isoform (11β-HSD1).

S100 proteins are a family of small calcium-binding proteins present in a wide variety of tissues. Specifically, in human skin, S100 is present in mainly antigen-presenting cells and myelinated cells as well as various tumours [16–19]. The antibody chosen for this experiment is an antibody for S100 beta. In the epidermis of the skin, the S100 antibody binds to Langerhans cells and melanocytes [19]. In the dermis of the skin, S100 binds to neuronal cells that are myelinated such as Ruffini endings, and Merkel disks as well as axonal cells in the cutaneous neural network [20]. Langerhans cells are differentiated dendritic cells that respond to skin irritation by releasing cytokines which is then felt as a painful sensation at the scar area [21]. Further, Langerhans cells have been shown to be correlated to the formation of hypertrophic scars [22,23]. The objective for using S100 is to investigate if AFL-CO_2 has an effect on the number of Langerhans cells within the scar, and to compare the results to the clinical outcomes of improved pain and itch [21–24].

11β-HSD1 is one of two isoforms of an enzyme located in the endoplasmic reticulum of cells in various tissues [25] and has the function of converting cortisone to cortisol/corticosterone [26] to provide the surrounding tissues with an endogenous cortisol supply. 11β-HSD1 studies have only been investigated in skin relatively recently [27]. In the skin, 11β-HSD1 is present in dermal fibroblasts, melanocytes, and keratinocytes [28,29]. The effect that this enzyme has on the surrounding cells is largely due to the cortisol it produces, namely anti-inflammatory properties and affects the proliferation of keratinocytes [26]. Glucocorticoids such as cortisol are used as topical treatments for burns and so it is hypothesised that the release of endogenous cortisol will help with the wound healing process. Local activation of 11β-HSD1 has been shown to have varied effects on the skin [29–32]. Increase in glucocorticoids in the skin decreases the proliferation of keratinocytes and fibroblasts, which has a positive effect on the clinical outcome for a hypertrophic scar. Glucocorticoids also decrease inflammation in and around the scar leading to more effective healing. And thirdly, the skin integrity as a result of glucocorticoids present benefits the scar healing process as it disrupts the tightly packed collagen bundles to allow ground substance to infiltrate in [26,29–32].

Thus, expression of S100 and 11β-HSD1 in the skin was investigated in relation to laser therapy treatment to shed more light on the mechanisms surrounding scar healing from laser therapy.

Based on clinical experience with the AFL-CO_2 technology, we expected a decrease in S100 and an increase in 11β-HSD1 in the skin which would indicate the therapy improves both scar healing and subjective patient outcomes.

2. Materials and methods

2.1. *Data collection*

All patient data and skin tissue biopsies were collected at the Burns Unit of Concord Repatriation General Hospital (CRGH) in Sydney, Australia, a state-wide referral centre for burn patients and a teaching hospital of the University of Sydney, under a well-established protocol [3] and validated ethics approval (CH62/6/2014–187, CH62/6/2017–008). Eligibility for the study included patients with hypertrophic scars after burn injuries with the ability to provide informed consent. Exclusion criteria included any impairing psychiatric or medical co-morbidity prohibiting the provision of informed consent [3,5,33]. Each individual underwent tissue collection of 3 mm punch biopsies at three different time points under local or general anaesthesia:

a) One biopsy of healthy skin in the vicinity of the scar & one biopsy of the scar tissue itself before the first treatment.
b) One biopsy of the scarred skin immediately before the second treatment.
c) One biopsy from the treated scar area at the last follow-up appointment.

The setting for the AFL-CO_2 laser treatment was chosen as previously described [3] using the 10,600-nm AFL-CO2 Ultrapulse (by Lumenis®), including ActiveFx™ (80–125 mJ Energy, 3–45% Density, 250–300 Hz Rate), DeepFx™ (15–50 mJ Energy, 5–15% Density, 300 Hz Rate) hand pieces and the SCAAR Fx™

Table 1 – Cohort data of patients in study. Includes median and range of relevant data.

Patient	Age	Interval from injury to first treatment (Days)	Number of treatments	Median Treatment Interval (Days)	Skin Type (Fitzpatrick)	TBSA (%)	Scar location (s)
1	67	333	4	161	3	-	Upper Limb
2	20	1089	4	216	3	25	Face, Upper Limb
3	58	380	3	226	4	15	Chest
4	47	595	5	70	3	4	Lower Limb
5	44	535	2	227	4	80	Face
6	32	758	4	260	6	41	Upper Limb
7	73	938	5	124	4	15	Chest, Upper Limb
8	19	489	6	140	3	12	Upper Limb
9	34	8903	3	270	2	56	Upper Limb
10	19	449	6	217	2	67	Face, neck, Upper Limb
11	29	5131	2	861	4	80	Upper Limb, Chest
12	57	345	6	91	5	22	Lower Limb
13	52	765	4	112	3	20	Upper Limb
Summary: Median (Range)	44 (19–73)	595 (333–8903)		216 (70–861)	3 (2–6)	23.5 (4–80)	

mode (60–150 mJ Energy, 1–5% Density, 250 Hz Rate). Settings were adjusted to each patient according to patient and scar characteristics (Fitzpatrick skin type, scar quality, thickness, maturation status, etc.). Single pulses were used without overlapping.

2.2. Cohort

Samples were taken from 13 patients. Their cohort data is described in Table 1.

2.2.1. Immunohistochemistry with S100 and 11β-HSD1

All tissue samples were fixed in 10% formalin for immunohistochemical analysis. The samples were processed, embedded in paraffin, and sectioned at 5 μm thickness. The tissue sections were deparaffinised in xylene and rehydrated prior to antigen retrieval in 10% DIVA Decloaker (DV2004MX, Biocare Medical) solution in a pressurised chamber heated to 80 °C for 15 min. Following washes with 0.015% bovine serum albumin, 0.01% Triton in phosphate buffered saline (PBS/BSA/Triton) and serum activity blocking with PBS/BSA/Triton with 5% goat serum, the sections were incubated overnight at 4 °C with primary human S100 antibody (ab868) and 11β-HSD1 antibody (ab169785) in 2% goat's serum in PBS/BSA at 1:200 and 1:100 ratios, respectively. Samples were subsequently incubated with secondary antibody solutions (0.5% S100/11β-HSD1 secondary IgG antibody with PBS/BSA in 2% goat serum) prior to staining with avidin-biotin complex reagent and DAB. Tissue sections were counterstained with 1:10 Harris' Haemotoxylin before microscopic examination and image analysis.

There are multiple crucial characteristics (genetics, ethnicity, presence of co-morbidities, smoking, nature and treatment of the burn wound etc.) which vary substantially from patient to patient and are believed to be essential factors contributing to the development of scars. Due to these inter-individual variations, adjacent non-scarred healthy tissue samples served as a control.

2.3. ImageJ analysis

All images were analysed using ImageJ Software with the Fiji plugin [34]. Adapted from a previously described protocol [33], samples were quantified using two methods; 1) Conversion to binary images with ImageJ and 2) scoring cells present using microscopy. The colour in the images was deconvoluted as to separate out the brown DAB stain from the rest of the image (Fig. 1B) from the original image (Fig. 1A). Using the threshold function in ImageJ, this was then turned into a black and white image (Fig. 1C) then quantified expression per area. The first method was used for the epidermal expression of 11β-HSD1 as individual cells were not counted. For the second method, cells were counted by two independent observers based on their histological morphology and brown pigment of the DAB stain using light microscopy. Fibroblasts stained by 11β-HSD1, and neuronal cells stained by S100 were identified by a brown coloured cells residing in the dermis. Langerhans cells were identified as brown cells in the epidermis. Any brown staining cells in the basement membrane were excluded as these represent

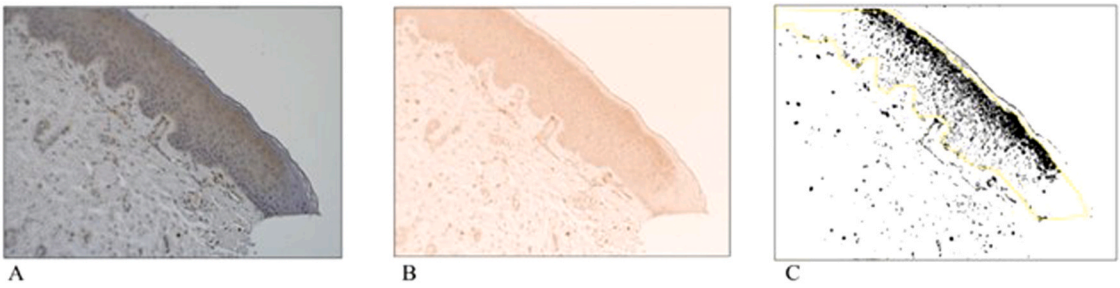

Fig. 1 – ImageJ with Fiji Plugin representation of the steps required for calculating 11β-HSD1 expression quantification in the epidermis; without modification (A), after colour deconvolution (B), and after threshold applied (C).

melanocytes. The cell counts were normalised per area, either in the epidermis or the dermis.

2.4. *Clinical scar assessment measures*

Clinical scar parameters were obtained from the patient database, corresponding to the histological samples analysed. All scars were assessed using the POSAS [35], the VSS, scar thickness measured with an ultrasound, the Douleur Neuropathique 4 Questionnaire (DN4), the 4-D Pruritus Scale, as well as the BSHS-B quality of life questionnaire (as previously described by our research group) [3].

To ensure that the same reference point was assessed, the scar(s) were photographed at the initial assessment, mapped out and the thickest point marked on the photo with an X. Each assessment was completed at the same anatomical location using the photographed reference.

Our aim was to compare histological findings with the scar assessment measures; assessing the correlation between markers of itch and pain (S100 protein), with measures including 4-D pruritus scale and the DN4 questionnaire and 11β-HSD1 levels in the dermis and epidermis as a measure of scar improvement, in correlation with assessment measures of POSAS, BSHS-B and VSS.

2.5. *Statistical analysis*

One-way ANOVA was performed using GraphPad Prism. All p values ≤ 0.05 are treated as statistically significant. Individual values were excluded using Grubb's test in GraphPad Prism. Data is expressed in mean ± SEM for the histological analyses.

3. Results

3.1. *Immunohistochemical results*

No significant difference was found in Langerhans cell populations in the epidermis over the course of the AFL-CO_2 therapy. Prior to treatment, a mean of 5/100,000 μm^2 of Langerhans cells were quantified in scar tissue, similar to 7/100,000 μm^2 Langerhans cells found in normal, healthy tissue. However, after treatment there was an increase to 7/100,000 μm^2 Langerhans cells. The 2nd AFL-CO_2 treatment

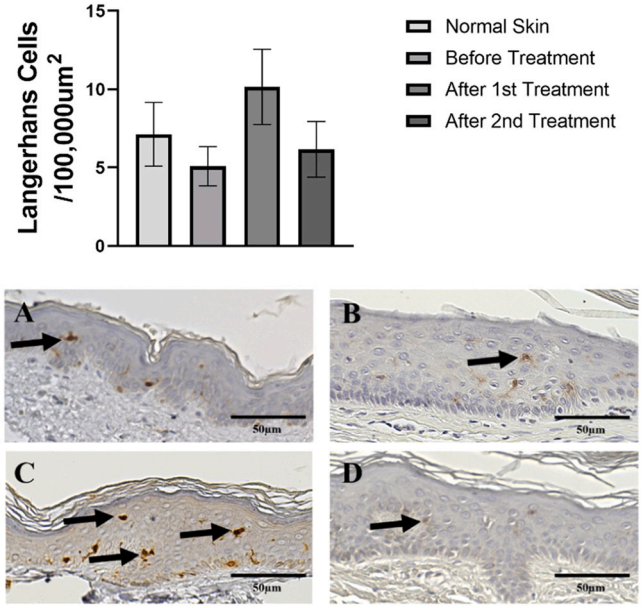

Fig. 2 – Immunohistochemical analysis of Langerhans cells in epidermis of human skin biopsies from patients at key intervals of treatment (n = 7–8). Graph: Langerhans cells stained by S100 quantification per 100,000 μm^2. Images: Langerhans cells (arrows) in normal skin (A), in scar tissue before 1st treatment (B), in scar tissue after 1st treatment (C) and in scar after 2nd treatment (D).

resulted in a 39% decrease in Langerhans cells compared to the 1st AFL-CO_2 treatment and only a 13% decrease overall (compared to normal skin) (Fig. 2). Neuronal cells were stained in the dermis of skin and scars using S100 and there was no statistically significant change over the course of the AFL-CO_2 therapy, but a general trend was observed. Prior to treatment, 2/100,00 μm^2 of neurons were measured which increased to an average of 4/100,00 μm^2 after the first treatment. Through successive treatment however, the number of neurons present decreased back down to a level similar to that found in normal skin (Fig. 3).

11β-HSD1 was expressed in the keratinocytes in the epidermis of skin and scars. There was a statistically significant increase after AFL-CO_2 therapy compared to before AFL-CO_2 therapy ($p < 0.01$) (Fig. 4), a mean percentage area expression

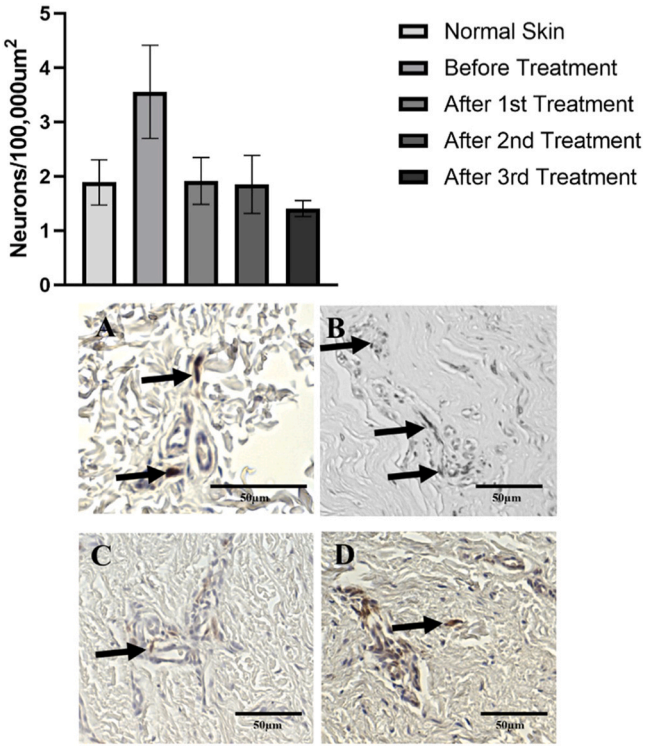

Fig. 3 – Immunohistochemical analysis of neurons in dermis of human skin biopsies from patients at key intervals of treatment (n = 3–7). Graph: Dermal neurons stained by S100 quantification per 100,000 µm^2. Images: Dermal neurons (arrows) in normal skin (A), in scar tissue before 1st treatment (B), in scar after 1st treatment (C), and scar tissue after 2nd treatment (D).

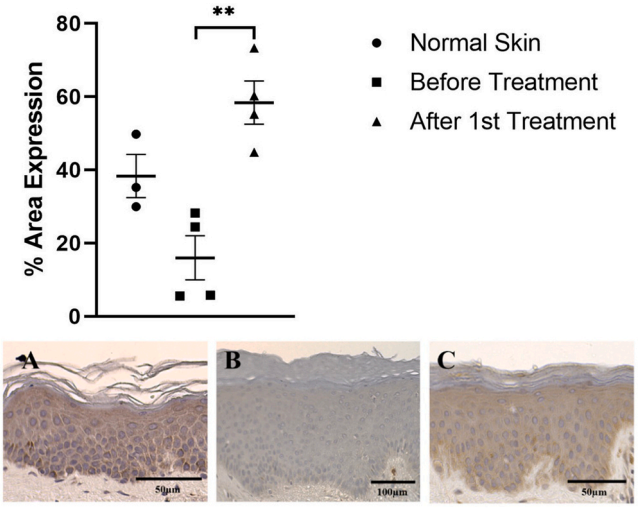

Fig. 4 – Immunohistochemical analysis of 11β-HSD1 expression in epidermis of human skin biopsies from patients at key intervals of treatment (n = 3–4). Graph: Expression of 11β-HSD1 as a percentage of epidermal area. Images: Epidermal 11β-HSD1 expression in normal skin (A), in scar tissue before 1st treatment (B), in and scar tissue after 1st treatment (C). ** $p < 0.01$.

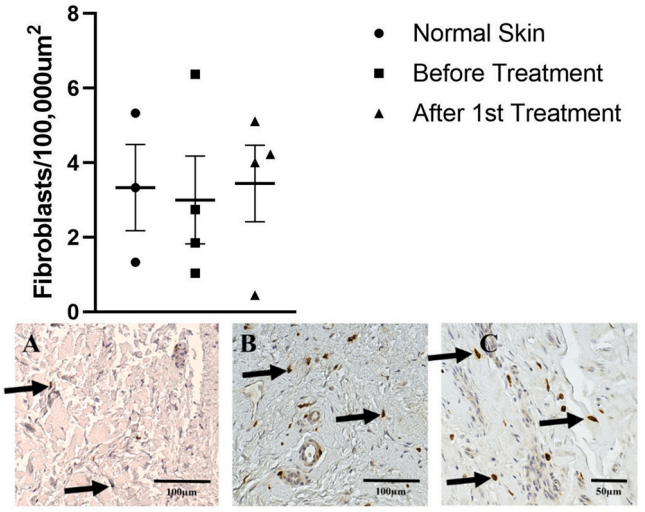

Fig. 5 – Immunohistochemical analysis of dermal fibroblasts of human skin biopsies from patients at key intervals of treatment (n = 3–4). Graph: Dermal fibroblasts stained by 11β-HSD1 quantification per 100,000 µm^2. Images: Dermal fibroblasts (arrows) in normal skin (A), scar tissue before 1st treatment (B), in and scar tissue after 1st treatment (C).

from 16% to 59% of the epidermis. Compared to normal skin, the 2nd treatment of AFL-CO_2 resulted in a 34% relative increase. No significant difference was found in fibroblast populations in the dermis over the course of the AFL-CO_2 therapy. Prior to treatment an average of 3/100,000 µm^2 fibroblasts were expressed in the dermis of skin and scars which only increased by 0.5/100,000 µm^2 after the first round of AFL-CO_2 therapy. (Fig. 5).

3.2. *Scar assessment*

The BSHS-B was the only assessment where a higher score is better, the other 5 record a higher score for a more severe pain or worse outcome as measured by their individual sections and hence an improvement in patient's outcome measures is represented by a decreased score. The 6 assessment surveys from each individual revealed mixed results (Fig. 6).

The POSAS-O, POSAS-P and VSS showed a significant decrease from the initial follow-up to the 3rd follow-up consultation with an average of 6.1–3.6 ($p < 0.001$), 8.3–5.6 ($p < 0.001$) and 9.0–6.2 ($p < 0001$), respectively. VSS significantly decreased from 8.9 to 6.7, 6.4 and 6.2 for the 1st, 2nd, and 3rd follow-up consultations ($p < 0.005$), respectively.

Burn specific quality of life improved from an average initial BSHS-B score of 110–120 after 1 treatment with the AFL-CO2, but returned to 110 at the 3rd follow up.

Pruritus and DN4 Pain scores both had a general trend of average initial scores (14.9 and 3.8 respectively) decreasing to

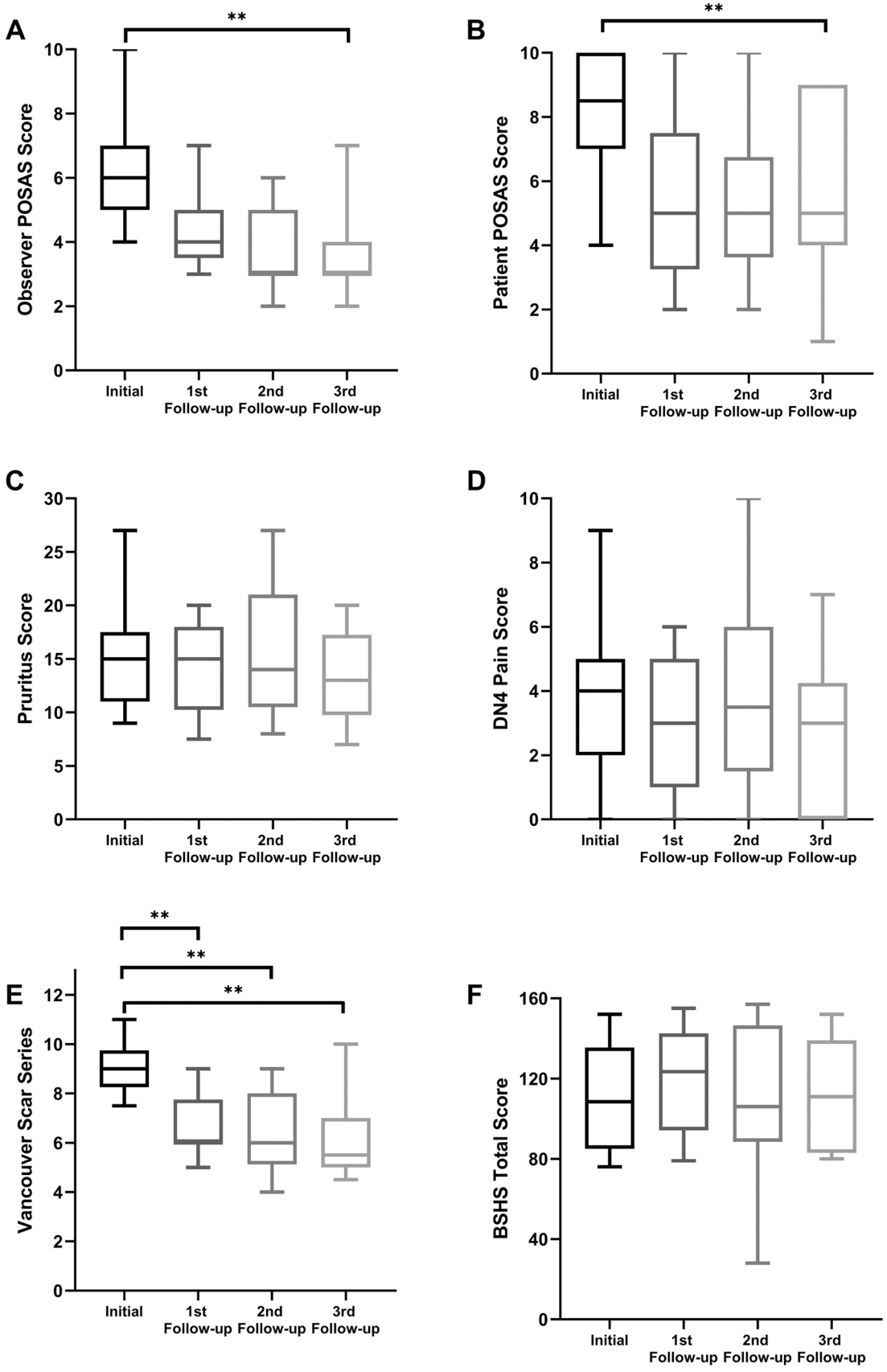

Fig. 6 – Boxplots illustrating the effect of AFL-CO_2 Therapy on objective and subjective impact on burn scar assessment from the initial assessment through to the 3rd follow-up assessment (n = 4). Plots represent Observer (A) and Patient (B) POSAS scores, Pruritus score using the modified score (C), DN4 Pain Score (D), Vancouver Scar Series Score (E), and the BSHS Scar Score (F). ** $p < 0.01$.

Table 2 – Summary table comparing immunohistochemical analyses (A) and scar assessment scores (B) displayed as median with interquartile range (IQR).

(A) Immunohistochemical Analysis	Normal Skin	Before Treatment	After 1st Treatment	After 2nd Treatment	After 3rd Treatment
Langerhans Cells*	6.5 (1.3–12.6)	4.8 (3.0–7.33)	11.1 (4.5–13.9)	7.3 (2.7–8.5)	
Neurons*	1.6 (1.1–2.6)	3.0 (1.7–5.8)	1.7 (1.1–2.2)	1.6 (1.1–2.9)	1.6 (1.1–1.6)
Epidermal expression of 11βHSD-1†	35.2 (30.0–49.8)	15.1 (5.6–27.3)	57.7 (47.4–70.0)		
Fibroblasts*	3.3 (1.3–5.3)	2.3 (1.2–5.5)	4.1 (1.3–4.9)		
(B) Scar Assessment Scores		**Initial**	**1st Follow Up**	**2nd Follow Up**	**3rd Follow Up**
POSAS-O overall (0–10)		5.5 (4.1–6.9)	3.8 (3.1–5.9)	3.3 (3.0–4.6)	3.3 (2.3–4.3)
POSAS-P overall (0–10)		7.0 (4.8–9.3)	5.5 (5.0–9.0)	5.5 (2.8–6.8)	5.0 (1.8–6.0)
Modified D4 Pruritus Questionnaire (7–35)		14.0 (9.8–16.8)	15.5 (11.8–19.3)	20.0 (14.0–27.0)	13.0 (10.0–16.0)
DN4 Pain Questionnaire (0–10)		3.5 (1.5–4.8)	4.0 (1.75–5.5)	4.5 (2.3–9.0)	3.5 (3.0–4.0)
VSS (0–13)		9.5 (8.6–10.0)	6.5 (5.3–7.4)	6.0 (6.0–7.5)	6.0 (5.3–7.5)
BSHS-B Total Score (0–160)		104.0 (90.0–130.8)	126.0 (121.0–132.0)	120.5 (146.5–45.0)	126.0 (113.3–137.3)

*Cells counted per 100,00 μm^2 †%Area expression.

the 3rd follow-up being the lowest average scores (13.5 and 2.6).

No distinct trend was found in the correlation between immunohistochemistry analysis and scar assessment scores. Table 2 displays the summary results of the immunohistochemistry analysis presented as median and interquartile range (IQR) in comparison to scar assessment scores. Categories of treatment and follow up are aligned according to the time at which the measurements were taken for ease of comparison. Median count of Langerhans cells, neurons, and fibroblasts, as well as 11β-HSD1 expression follows the same trend. They are presented as median in the table for direct comparison to the median scar assessment scores.

4. Discussion

4.1. *Immunohistochemistry analysis*

The present study indicates that the markers chosen, reveal a more complex process underlying AFL-CO_2 that cannot be understood just by investigating the effects mediated by 11β-HSD1 or S100 as few statistically significant changes were seen in the expression of these molecules during treatment.

The change in the number of Langerhans cells was inconclusive in the relationship towards laser therapy (Fig. 3). In looking at epidermal and dermal expression simultaneously however, there is an observable trend, namely, that progressive laser therapy decreases the expression of cells containing S100. As S100 stains for a variety of nerve cells in the skin, this may be an indication that the pain and itchiness sensation decreasing after laser therapy is explained by decreasing S100 levels in the skin [36]. The presented data suggests that, when comparing the decrease of Langerhans cells and neurons following subsequent treatments which parallels the decrease in Pruritus and DN4 questionnaire scores at the 3rd follow up (Table 2). This result is helpful for future research as it indicates a more complex relationship between the laser therapy and cutaneous nerve cells is present and therefore warrants further investigation perhaps using more specific neuronal protein markers for particular receptors.

Keratinocyte expression of 11β-HSD1 in the epidermis shows a distinct increase from before and after laser therapy (Fig. 4). However, dermal fibroblasts expression of 11β-HSD1 did not show the same marked trend. There was only a weak trend observed in most of the data points in Fig. 6 which does follow the same trend observed in the epidermis. This indicates that a more complex relationship between wound healing and 11β-HSD1 expression is present. Cell dependant activation of 11β-HSD1 might be an explanation by the disparity of the results. After AFL-CO_2 surgery was performed, topical glucocorticoids were applied to the scar and so the relationship between exogenous and endogenous cortisol presence could also have an effect on 11β-HSD1 activity in the epidermis. However, it seems as if the effect of endogenous cortisol in the skin may not play a significant role in the scar remodelling as there was no change in dermal 11β-HSD1, which is the histological site of collagen deposition.

4.2. *Correlation between clinical results and histological data gathered*

The two questionaries that directly assess pruritus and pain associated from scars are the DN4 and modified 4D itch scores. Although they do not exhibit a clear decrease in pruritus associated with ongoing treatment of AFL-CO_2 therapy, the decrease in the 3rd follow-up may point to either a delayed effect of the therapy or the additive effect of the therapy in decreasing scar pain. The unclear relationship may be explained by the similar relationship found in the analysis of S100 in the Langerhans and neuronal cells. Given that both the Langerhans and neuronal cells, and the DN4 and 4D itch score both were used as an indication of the patient's pruritus and that they give similarly unclear relationships, it may be indicative of a more complex underlying mechanism.

The sharp increase in 11BHSD1 expression after the 1st treatment can be correlated to the decrease in VSS score at the 1st follow-up after the 1st treatment. Even though VSS is not a questionnaire from the patient's perspective, the

physical properties of the scar assessed in the score is likely correlating to the enzyme expression in the skin. The explanation for the change in physical nature of the scar could be explained by the fact that the elevated levels of 11BHSD1 would probably imply an increase in endogenous cortisol and this would have an effect on the skin integrity as it begins to remodel and therefore physical presentation of the scar would be expected to change.

Both the Observer and Patient POSAS scores as well as the BSHS-B score indicate a decrease in pruritus and increase in overall QoL across a range of factors. The histological analysis alone does not give an indication of the improving nature of the scars and so both a longer study and more data would be necessary if any comparison could be drawn between the more comprehensive POSAS score with histological analyses.

4.3. *Laser therapies*

The popularity of laser-based burn scar revision has increased significantly over the past two decades and several different laser modalities described to manage pain and scarring of scars. Aside of the newer ablative fractional CO2 laser, the 585 nm and 595 nm pulsed dye laser (PDL) has probably been one of the most researched lasers for hypertrophic scarring in the literature with a positive effect [37–41].

Whilst the ablative fractional CO2 laser has been widely accepted and clinically became the gold standard for laser scar revision of burn scars, combined laser treatments are getting more and more popular with great results and described histological/molecular changes [42]. It will be interesting to investigate the effect of multimodal laser therapies on a molecular level in future studies.

4.4. *Limitations*

This study does present some limitations. IHC is only a semi-quantitative measure and so specific levels of proteins could not be accurately measured.

There were limitations associated with data collection for this study which thereby decreased the overall sample size of the pilot study. The driving factor behind the limited study size was due to the nature of the tissue samples. To ethically minimize the area of tissue taken from the scar and to minimize harm caused by taking multiple and/or larger size biopsies, the skin biopsies taken were very small. However, the small size of the biopsies had an unexpected significant impact on tissue processing. The small biopsies were more difficult to fix and section, reducing the pool of viable samples dramatically.

Nevertheless, given these limitations, the results found are promising and warrant further study.

4.5. *Future directions*

In consideration of the above limitations and being a pilot study, the nature of this investigation was to determine future avenues of potential studies, therefore proposals for further research will be explored. Further directions can be broadly categorised into two main approaches. The first of these is to further explore S100 and 11β-HSD1. Qualitative analyses such as investigating protein regulating through genomic relationships [43] could be used to understand if there is a protein regulation change as a result of AFL-CO_2 therapy, which would give a more accurate indication of changes in protein expression compared to IHC methods. Specifically regarding 11β-HSD1, the ratio of serum and/or skin concentration of cortisol and cortisone would be a more direct way to measure the effects of endogenous glucocorticoids on the skin and the changes before and after treatment.

The second approach for future directions would be to further explore the concepts of pain and pruritus by using different proteins as diagnostic tools. Among these could include more specific markers for dendritic cells in detecting Langerhans cell in the epidermis and further investigating the exact response of Langerhans cells to burn injury and AFL-CO_2 therapy. This could extend to other cells, and rather than looking at histological analyses, analysing cell to cell interactions at a smaller scale to ascertain exact pathways and thus determine the molecules and mechanisms involved.

Furthermore, as S100 did not detect non-myelinated nerves and since free-nerve endings play a large role in pain sensation [44] detecting these would give a more accurate indication of pain responses in individuals.

By collecting more data from further follow-up treatments and increasing the sample size, we would be able to generate a more accurate comparison of the histological data with the clinical outcomes.

5. Conclusion

In conclusion, there is no definitive link between the laser therapy and the markers S100 and 11β-HSD1 and their effects on the scarring. The expression of S100 in skin cannot be directly linked to the action of the AFL-CO_2 therapy and it cannot be concluded that the degrading of the collagen in the scars can be attributed to the endogenous production of cortisol, despite the promising results of the 11β-HSD1 expression in the epidermis. This pilot study warrants further investigations into relationships of other protein markers in the skin to hypertrophic scar formation and healing, and their subsequent link to AFL-CO_2 therapy.

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Declarations of interest

None.

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