# Evaluation of skin-surface interleukin 1 alpha, interleukin-1 Receptor Antagonist, CXCL-1/2 and beta-defensin-1 as non-invasive biomarkers for monitoring psoriasis vulgaris

**Kadri Orro** ( kadri.orro@fibrotx.com )
Tallinn University of Technology; FibroTx LLC https://orcid.org/0000-0001-5082-3097
**Kristiina Salk**
FibroTx
**Kristi Abram**
Clinic of Dermatology, Tartu University Hospital
**Jelena Arshavskaja**
FibroTx
**Anne Meikas**
TFTAK
**Maire Karelson**
Clinic of Dermatology, Tartu University Hospital
**Toomas Neuman**
FibroTx
**Külli Kingo**
Clinic of Dermatology, Tartu University Hospital
**Pieter Spee**
PS! Pharmaconsult

**Research**

**Keywords:** biomarker, Transdermal Analysis Patch, IL-1, IL-1RA, CXCL-1, CXCL-2, hBD-1, psoriasis, inflammation, dermatology, skin care diagnostics, treatment monitoring

**Posted Date:** June 16th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-33637/v1

## Abstract

# Background:

Psoriatic skin is typically evaluated through visual assessment of its clinical hallmarks: thickness, redness and scaling of skin lesions. Sonography is used to physically monitor disease severity through physical assessment of the skin outer layers. Both methods assess consequences of inflammation rather than the molecular basis of disease. The aim of the current study was to assess 1) whether expression patterns of IL-1α, IL-1RA, CXCL-1, CXCL-2, and hBD-1, examples of proteins known to drive psoriasis pathology, can be measured non-invasively from the skin surface, 2) whether expression patterns of these proteins correlate with disease severity, and 3) whether skin surface expression of these proteins can be used to measure pharmacodynamic effects of psoriasis treatment.

# Results:

Using non-invasive FibroTx TAP technology for sampling and measurements of proteins from the skin of psoriasis patients revealed clear differences of IL-1α, IL-1RA, CXCL-1/2 on lesional skin when compared to non-lesional skin or skin from healthy volunteers. Comparing these expression patterns with visual assessment of thickness, redness and scaling of skin lesions revealed no significant quantitative correlations, with the exception of a weak correlation between CXCL-1/2 and thickness of lesions. Similarly, there were no significant correlations between FibroTx TAP measurements and ultrasound measurements, with the exception of a weak correlation between CXCL-1/2 and SLEB thickness. The potential of these skin-surface biomarkers were studied by monitoring skin lesions of psoriasis patients undergoing narrow-band UVB (311 nm) phototherapy. During the course of UVB treatment, clear patterns towards normalisation of IL-1RA and CXCL-1/2 were observed on lesions measured.

# Conclusions:

Skin surface measurements of proteins involved in psoriasis skin pathology, in this study exemplified by IL-1α, IL-1RA, CXCL-1/2 and hBD-1, have potential as biomarkers for monitoring severity of disease, as well as for monitoring pharmacodynamic changes. Skin surface measurements of IL-1RA and CXCL-1/2 displayed a different profile than achieved by visual scoring of local inflammation or sonography, thus confirming that measuring the ‘molecular root’ of inflammation appears to have value as an objective, non-invasive biomarker measurement for scoring disease severity in its own right.

## Introduction

Psoriasis is a chronic relapsing immune inflammatory dermatosis with different clinical manifestations that affects 1–3% of the world population [1]. Psoriasis is most common in fair-skinned people and extremely rare in dark-skinned individuals [2]. Psoriasis causes itching in 60–70% of cases [3]. Plaque

psoriasis is the most common variant of psoriasis, characterized by inflamed erythematous lesions of the skin caused by the interplay between immune cells, keratinocytes and other skin-resident cells, mediated by adaptive and innate immune system components [4].

In psoriasis, a defective regulation of epidermal keratinocytes by dermal fibroblasts leads to hyper-proliferation of epidermal keratinocytes, which results in thickening of the skin and deposition of poor quality keratin, which causes scaling[5]. In addition, infiltrating neutrophils and T-cells contribute to clinical symptoms, such as swelling and redness, and cause a permanent state of chronic inflammation in affected skin[6]. Psoriasis patients often develop additional clinical symptoms or diseases, such as psoriatic arthritis, which develops in up to 24% of psoriasis patients[7]. Despite this knowledge, it remains difficult to predict both onset and progression of psoriasis, and there is an unmet medical need for methods that can be used to measure if people are at risk for psoriasis, and/or that can predict how disease progresses, both with respect to severity, and with respect to other clinical symptoms or diseases.

Clinical evaluation of psoriasis is primarily performed visually. The Psoriasis Area Severity Index (PASI) is a clinical score based on assessment of the percentage of skin affected (on head, trunk, arms and legs) and severity of local inflammation scores of the skin thickness, redness and scaling in these areas [8–10]. The PASI score allows monitoring of changes in affected skin areas over time, which may either reflect progression, relapse or improvement of the disease. However, PASI also has its limitations: it can be subjective and gross difference may occur between examiners; it can present poor sensitivity in small areas of involvement, being not sensitive enough for patients with mild disease.

Another method for non-invasive evaluation of psoriatic skin is sonography, a “real-time” imaging technique based on ultrasound measurements that allow assessment of the morphological and structural appearance of psoriatic skin lesions at the moment of diagnosis, but also allows monitoring of changes of the underlying tissue during therapy[11–13].

Treatment of chronic plaque psoriasis depends on the severity and areas affected in patients. Treatment options range from local ointments for mild psoriasis, to more harsh therapies for moderate and severe psoriasis, such as phototherapy (e.g. UVB), photo chemotherapy (e.g. PUVA), systemic treatment (e.g. methotrexate, cyclosporine, acitretin) or biological treatment (e.g. Humira, Stelara, Cosentyx) [14–16]. Therapeutic efficacy, defined as a diminishment in psoriasis clinical scores, does not occur instantly, and patients may not respond to therapy at all. At the moment, there are no methods in the clinic that can objectively predict response to psoriasis treatment and/or methods that can objectively measure therapeutic efficacy. Nonetheless, patients are treated with harsh immuno-suppressive treatments, and are put at risk for developing side effects, such as infectious diseases (biologic therapies), or an increased risk for skin cancer (PUVA, phototherapy)[17;18]. Hence, there is an unmet clinical need for methods that can objectively predict and/or measure response to therapy. This to provide medical doctor’s an improved method for risk-benefit assessment with respect to treatment options for patients.

A method comply such needs is a biomarker assessment. Biomarker or biological marker is identified as a biological characteristic that can be measured and evaluated objectively as an indicator of normal and pathogenic biological processes or pharmacological response to therapeutic treatment [19].

Biomarkers are attractive especially due to their predictive value. It has shown that the development of rheumatoid arthritis can be predicted months before clinical signs by the presence of cyclic-citrullinated peptide reactive antibodies in blood [20]. Portugal-Cohen has published a non-invasive method in which a limited number of soluble biomarkers could be assessed in skin-lavage from lesional skin of a limited number of psoriasis patients and renal failure [21–23]. A clear increased concentration of IL-1α, TNF-α and IL-6 was found in skin lavage from lesion sites of psoriasis patients, in comparison with skin lavage from non-lesional sites, or from skin of healthy individuals [23]. Malaviya et al, has shown that the amount of cleaved Caspase-3-positive cells predicts accurately response to inhibitor of TNF-α like etanercept, months before response to therapy can be assessed based on clinical symptoms [24]. Biomarkers that similarly predict onset and/or progression of psoriasis may be identified, as well. Dand *et al.* reported HLA-C*06:02 genotype as a predictive biomarker of biologic treatment response in psoriasis for Adalimumab (anti–TNF- α) and Ustekinumab (anti–IL-12/23) [25]. Several studies have identified biomarkers which correlate longitudinally with the history of the disease and skin conditions for psoriasis and atopic dermatitis, including biomarkers for keratinocyte activity (e.g. presence of K16) and inflammatory response (e.g. up-regulation of IL-1α and TNF-α) [20;24; 26–33]. Biomarkers which are surrogate endpoints for changes that predict clinical benefits for psoriasis and atopic dermatitis therapy are identified as well, including biomarkers representing Th17 pathway, monocyte activity (e.g. TNF-α lymphocyte activity (Granzyme B) and type I interferon pathways[18;20; 20; 24–33].

We have previously introduced a non-invasive method to measure biomarkers directly from skin[34]. The FibroTx Transdermal Application Patch (FibroTx TAP) consists of an adhesive bandage that contains a nitrocellulose insert on which specific antibodies have been printed for capturing proteins directly from the skin surface. Captured biomarkers are qualitatively and quantitatively analyzed using spot-ELISA, performed directly on the TAP insert.

To test the potential of skin-surface proteins for monitoring psoriasis severity and/or progression, we have studied IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on the skin surface of moderate-to-severe psoriasis patients using non-invasive FibroTx TAP technology. In the study, we have compared presence of these proteins on lesional and non-lesional skin with ultrasound measurements and with clinical inflammation scores for psoriasis activity and severity. Subsequently, we have measured whether skin surface IL-1α, IL-1RA, CXCL-1/2 and/ or hBD-1 can monitor treatment effectiveness during narrow band ultraviolet B therapy, to test their potential as candidate biomarkers for monitoring disease/ for treatment response.

# Materials And Methods

# Study participants

The study was performed at the Dermatology Clinic of Tartu University Hospital in Estonia, under the approval of Tallinn Medical Research Ethical. In total 44 patients with plaque psoriasis and 10 healthy volunteers were included in the study. Patients with mild to severe *psoriasis vulgaris* visiting dermatologist at Tartu University Hospital Dermatology clinic were included to the study. Prior the study, the detailed aim of the study was explained to each of the volunteer and an informed consent to participate was signed voluntarily by each of patient. Local skin status and the severity of the psoriasis were assessed according to the degree of scaling, erythema, thickness and PASI score by the same dermatologist during the visit.

Patients included in the study had not received any systemic form of medical treatment and all kind of phototherapies for at least 4 weeks prior to study and have not received any topical form of medical treatment for at least 2 weeks prior to study. Pregnant or breastfeeding women and volunteers with a history of other skin diseases were excluded from participation.

# Antibodies

Human GRO-ß (CXCL-2) ELISA Development Kit (Cat. No: 900-K120, PeproTech), Human IL-1α ELISA Development Kit (Cat. No: 900-K11, PeproTech), Human hBD-1 ELISA Development Kit (Cat. No: 900-K202, PeproTech), Human IL-1RA ELISA Development Kit (Cat. No: 900-K474, PeproTech).

# TAP biomarker measurements from skin

Biomarker measurements from skin were performed using FibroTx TAP capture antibody micro-arrays containing three spots of biomarker capturing antibody: 0.25 ng of IL-1α, 2.25 ng IL-1RA, 2.25 ng of CXCL- 1/2 and 2.25 ng of hBD-1 per spot, additionally each micro-array contained a negative control (PBS-with 20% (v/v) glycerol) and positive control (0.03 ng biotinylated anti- hBD-1). FibroTx TAP capture antibody micro-arrays coated with anti-IL-1α, anti-IL-1RA, -anti CXCL-1/2 and anti-hBD-1 were applied to the normal appearing and lesional skin of psoriasis patients (N = 44) and onto the skin of healthy volunteers (N = 10). FibroTx TAP capture antibody micro-arrays were incubated on skin for 20 minutes. Following incubation, FibroTx TAP capture antibody micro-arrays were removed from the skin and stored at 4 °C until further analysis. Captured IL-1α and IL-1RA, CXCL-1/2 and hBD-1 were visualised using spot-ELISA, as previously described [34].

# Ultrasound measurements

Determination of differences in thickness of skin layers (epidermis, sub-epidermal low-echogenic band (SLEB) and dermis) between non-lesional and lesional skin on psoriasis was carried out using DermaLab Combo SkinLab from Cortex Technology according to manufacturer's instructions. Ultrasound imaging was conducted from the exact same skin area as FibroTx TAP measurements, after TAP removal from non-lesional and lesional skin.

# Narrow-band UVB treatment

The narrow-band UVB-treatment by minimal erythema dose MED protocol [35] was performed in Tartu University Hospital Dermatology Clinic. For inclusion requirement a 4-week wash-out period for systemic and all kind of phototherapy and 2 weeks of topical treatment was set. In total 14 patients with psoriasis were enrolled for narrow-band UVB treatment 3 times a week, 30 treatments were made all together during the 8 weeks. All patients gave prior written informed consent for the study. Treatment schedule, possible benefits and side effects of treatment was explained to all patients. Biomarker measurements with FibroTx TAP where performed before the first treatment (serves as base line) and after the second week and fourth week of treatment.

# Statistical analyses

Statistical calculations were performed using freeware statistics program JASP (version 0.9.2 for macOS). Statistical significance of numerical variables of two unrelated groups were determined with two sample independent t-test, for related groups analysis paired sample t-test was used. For correlation analysis the normality of the data was tested with the Shapiro-Wilk test followed by Spearman's Rank correlation analysis, statistical significances were verified with probability value (*p*-value).

# Ethical considerations

Ethical approval for the studies is covered by Decision No. 2551 from the Tallinn Medical Research Ethical Committee. Participants data has been collected such that it cannot be traced back directly to patients by FibroTx employees.

## Results

# FibroTx TAP protein measurements on lesional skin compared to non-lesional skin and healthy volunteers

The skin-surface expression of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 was measured on lesional skin (L) and normal appearing skin (NL) of psoriasis patients (N = 30), and on skin of healthy individuals (HV) (N = 10), using FibroTx TAP tests. Statistically significant differences were observed between measurements of IL-1α, IL-1RA, CXCL-1/2 on lesional skin and non-lesional skin of psoriasis patients, depicted on paired (Fig. 1A, panel A-D) and unpaired (Fig. 1A, panel E-H) data analyses, but not for hBD-1. The patterns of IL-1α, IL-1RA, CXCL-1/2 measurements were largely consistent for all patients, but substantial variations were found in expression levels of individual proteins on lesional and non-lesional skin amongst single patients (Fig. 1A, panel A-D).

The levels of IL-1α found on lesional skin were significantly lower than levels found on normal appearing skin; a pattern that was observed in 24 of 30 psoriasis patients ($p < 0.01$, Fig. 1A, panel E). In contrast, the levels of IL-1RA and CXCL-1/2 detected on lesional skin were significantly higher compared to levels found on normal appearing skin of psoriasis patients ($p < 0.001$ and $p < 0.01$, respectively); a pattern that was observed in 26 and 17 of 30 psoriasis patients for IL1-RA and CXCL-1/2, respectively (Fig. 1A, panels

F and G). The expression levels of hBD-1 found on lesional skin of psoriasis patients were similar to the levels of hBD-1 found on normal appearing skin of psoriasis (Fig. 1A, panel H). Biomarker levels detected on healthy apparent skin of psoriasis patients appeared similar to the levels captured on the skin of healthy individuals (Fig. 1B, panel A-D). Thus, there is a clear correlation between expression levels of IL-1α, IL-1RA, CXCL-1/2 (but not hBD-1) and the condition of skin in psoriasis patients.

The inverse expression patterns of IL-1α and IL-1IL-1RA on lesional and non-lesional skin of psoriasis patients, as well as the biological link between IL-1α and IL-1RA, prompted us to analyse the molar ratio between IL-1α and IL-1RA on lesional and non-lesional skin of psoriasis patients. IL-1α and IL-1RA bind to the same receptor, the IL-1 receptor (IL-1R), as a pro-inflammatory agonist and an anti-inflammatory antagonist, respectively. Two forms of IL-1α exist, the immature form with a MW of 31 kDa, and the mature form of 18 kDa, that are both biologically active [36]. Both isoforms are recognised by antibodies used for FibroTx TAP. IL-1RA is predominantly expressed as a 17.1 kDa protein [37]. The analyses revealed that there is clear molecular excess of IL-1α over IL-1RA on non-lesional skin of psoriasis patients and skin of healthy volunteers, regardless whether IL-1α is present in immature or in mature form, or a combination there-of. Similarly, there is a clear excess of IL-1RA over IL-1α on lesional skin of psoriasis patients regardless of the form of IL-1α (see Table 1).

# Correlations between FibroTx TAP protein measurements and psoriasis clinical scores

IL-1α, IL-1RA, CXCL-1/2, are cytokines directly involved in plaque psoriasis skin-inflammation [38;39], which visually manifests itself in the form of redness, thickness and scaling of affected skin [4, 8]. A possible explanation for the large differences in skin-surface levels of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on lesional skin of individual patients may be differences in disease severity between patients. A clinical method to assess psoriasis severity is the PASI score, which is a weighted score comprised of body surface area affected (BSA) combined with redness, thickness- and scaling of the skin, measured in four different areas of the body (head, trunk, arms, legs) [8;9]. To assess correlations between FibroTx TAP measurements of psoriatic skin and elements of the PASI score, we analysed the correlation between the values of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 measurements from psoriatic skin against the values (0–4 scale) of thickness, scaling and redness at the area of FibroTx TAP measurements, as assessed by a dermatologist. The only statistically significant correlation found was a positive correlation between the clinical score for thickness and levels of CXCL-1/2 on lesional skin (see Fig. 2 and Table 2).

No statistically significant correlations between measurements of either IL-1α or IL-1RA and any of the clinical scores were detected. Nevertheless, a tendency towards a negative correlation between FibroTx TAP measurements of IL-1α and scaling was observed. The higher the levels of IL-1α on psoriatic lesions, the lower the scaling of lesions (see Fig. 2). No apparent correlations were found for FibroTx TAP measurements of hBD-1 from psoriatic skin and clinical assessments of redness, thickness and scaling.

**Correlations between FibroTx TAP protein measurements and ultrasound analysis on skin.**

Plaque psoriasis manifests itself in physical changes of the skin layers, such as thickening of the epidermis and presence of a characteristic low-density layer between epidermis and dermis, the so-called sub-epidermal low-echogenic band (SLEB), that can be measured via ultrasound [11–13].

To determine whether differences in the molecular expression patterns of IL-1α, IL-1RA, CXCL-1/2 and hBD-1, between non-lesional and lesional skin site of psoriasis patients correlate with alterations in physical properties of skin layers, FibroTx TAP measurements of these four proteins were correlated with ultrasound measurements from exactly the same skin of psoriasis patients. Using ultrasound, a clear and statistically significant thickening of epidermis ($p < 0.01$), SLEB ($p < 0.001$) and dermis ($p < 0.001$) was measured in lesional skin of psoriasis patients in comparison with non-lesional skin from the same patients (see Fig. 3 panel A - C).

Combining FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 and ultrasound measurements of normal appearing skin from the same patients did not reveal any strong significant correlations between expression of IL-1α, CXCL-1/2 or hBD-1 and thickness of the epidermis, dermis or SLEB (see Table 3A). A mild positive correlation between IL-1RA and SLEB thickness was observed on non-lesional skin, but not between IL-1RA and epidermis or dermis thickness of lesional skin sites. Combining FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 and ultrasound measurements of lesional skin from the same patients also did not reveal any significant correlations between expression of IL-1α, IL-1RA or hBD-1 and thickness of the epidermis, dermis or SLEB (see Table 3B).

The only positive correlation was observed between CXCL-1/2 and SLEB thickness on lesional skin. No such correlation was noted between the expression of the CXCL-1/2 and epidermis nor dermis thickness.

To assess whether there is correlations between clinical scores and the ultrasound measurements clinical inflammation scores where correlated with thickness of epidermis, dermis and SLEB analysed at the same skin site. No correlation between skin layer parameters and PASI score was observed whereas positive correlation between lesional skin SLEB thickness and clinical scores of thickness assessed ($p < 0.05$) by doctor was detected (see Table 3C). Among psoriasis clinical scores themselves strong positive correlations between PASI and skin thickness ($p < 0.01$) as well as between PASI and redness was detected ($p < 0.01$).

# Response to narrow-band UVB treatment measured with TAP

The significant differences between expression levels of IL-1α, IL-1RA, CXCL-1/2 on normal appearing skin and lesional skin of psoriasis patients suggest a strong correlation with disease. Then again, there are no significant correlations between levels of IL-1α, IL-1RA and hBD-1 on skin and quantitative assessments of disease intensity and severity in the form of PASI or ultrasound measurements with the exception of CXCL-1/2 presenting mild positive correlation between skin thickness and SLEB on lesional skin. To assess whether IL-1α, IL-1RA, CXCL-1/2 and hBD-1 are merely qualitative markers of disease,

rather than quantitative, we measured expression levels of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 from the skin surface of 14 psoriasis patients undergoing whole-body treatment with narrow band ultraviolet B. FibroTx TAP was used to measure expression of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on lesional and normal appearing skin of patients before treatment initiation, after two weeks and after four weeks of treatment. Treatments were typically performed on three consecutive days in a week. To minimise the risk of measuring UVB-induced inflammation in the skin, rather than measuring the therapeutic effects of UVB on disease, all FibroTx TAP and clinical measurements were performed four days after treatment right before the start of a new three-day UVB-treatment cycle. Measurements were performed on exactly the same position on skin on each time-points. In parallel, visual scores for local inflammation (redness, thickness and scaling) were performed at the exact location of TAP measurements. In addition, the PASI score was determined before and after four weeks of treatment.

As a result of the narrow-band UVB treatment, the PASI score dropped on average 57.71 percent during treatment, a difference that was highly significant ($p < 0.001$) (see Fig. 4A, panel A). Scores for local inflammation (thickness, scaling and redness) showed highly significant improvements of the lesions measured by FibroTx TAP ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively) (see Fig. 4A panel B - C). During the four weeks course of UVB treatment, levels of IL-1α did not change on lesional skin of psoriasis patients, but there was a modest decline in IL-1α on normal appearing skin. In contrast, levels of IL-1RA ($p = 0.004$) and CXCL-1/2 ($p = 0.012$) showed a significant reduction on lesional skin in response to narrow-band UVB treatment. Whereas four weeks of treatment reduced IL-1RA on lesional skin to the level of IL-1RA on normal appearing skin before treatment, CXCL-1/2 showed a 75 percent reduction of the levels of CXCL-1/2 observed on lesional skin before treatment. No alterations were measured for IL-1RA on normal appearing skin during the course of treatment and CXCL-1/2 remained undetectable. Analyses of the IL-1RA over IL-1α ratio also confirm the clinically observed pattern of normalisation of skin in lesions measured. The ratio between IL-1RA and IL-1α, measured on lesions in apparent concentration (ng/ml) declined from 4.91 to 2.25. In contrast, the ratio between IL-1RA and IL-1α, measured on non-lesional skin remained fairly stable, changing from 0.57 to 0.65 during treatment (see Table 4).

The levels of antimicrobial peptide hBD-1 detected at base line on non-lesional skin are nearly 2-fold lower compared to the levels captured on lesional skin, however after 4 weeks of narrow-band UVB treatment the levels of hBD-1 detected on non-lesional skin are increased compared to the baseline approximately 2-fold contrary to the levels of hBD1 captured on lesional skin where nearly 4-fold decrease compared to amounts of base line hBD-1 is detected. Due to the UVB treatment the ratio of hBD-1 detected on healthy apparent and lesional skin at base line has changed opposite after 4 weeks.

# Discussion

To improve psoriasis care, diagnostic methods are needed that can facilitate personalized medicine. Such diagnostic method should be objective, accurate, cost-effective and easy-to-use for both patients and health-care professionals. Proteins, such as interleukins, chemokines, cell surface receptors and anti-microbial peptides drive the biological processes underlying both the physical and visual hallmarks of

psoriatic skin. As such, this psoriasis 'molecular footprint' may be very suitable for the development of diagnostic methods that can monitor disease progression, as well as measure response to treatment. Particularly suitable may be proteins that can be assessed non-invasively from the skin surface (i.e. without disrupting the skin). A prerequisite is that skin surface molecules follow the state of disease like. Therefore, the primary aim of this study was to assess whether expression patterns of proteins known to be involved in psoriasis, and that can be measured non-invasively from the skin surface, correlate with physical and visual hallmarks of psoriatic skin.

The panel of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 was arbitrarily chosen based on their role psoriasis, as reported in the literature, as well as because these proteins can be measured from the skin surface. IL-1α and IL-1RA are examples of a pro-inflammatory and anti-inflammatory interleukins, respectively, that are known to play important roles in skin homeostasis and skin inflammation, including psoriasis [36–40; 43]. The combination of chemokines CXCL-1 and – 2 was chosen because of their roles in attracting neutrophils to psoriatic skin lesions [38, 41]. The anti-microbial peptide hBD-1 was chosen as a representative of beta-defensins in psoriasis [44, 45]. The choice for measuring skin surface proteins using FibroTx TAP was based on the fact that FibroTx TAP is a non-invasive sampling technology that does not affect skin, i.e. protein measurements are not biased by skin responding to the measurement method, and do not interfere with biological processes in and on the skin [40]. To underline this, no adverse events were reported during FibroTx TAP measurements, neither on normal appearing skin nor on lesional skin, neither in patients nor in healthy individuals were reported, neither by visual assessment (e.g. signs of redness) or upon inquiry (e.g. irritation, itching, pain).

Expression patterns of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on the skin surface, as measured by FibroTx TAP, reflect reported protein expression patterns as assessed by more invasive technologies, such as mRNA analyses and immuno-histochemistry (IHC) using skin biopsies and protein-analyses after tape-stripping of the stratum corneum [38, 40, 42– 45]. It appears thus that protein expression in the skin is reflected both qualitatively and quantitatively on the skin surface.

Importantly, the fact that we find some proteins, like IL-1RA, CXCL-1/2 and hBD-1, are present in higher amounts on lesional skin, whereas others, like IL-α, are found in reduced amounts on lesional skin in comparison with non-lesional and healthy skin, indicates that differences in proteins measured cannot simply be attributed to e.g. differences in skin texture, skin barrier function or amounts of dead cells on lesional skin. Instead, these differences rather indicate that amounts of proteins found on skin reflect regulation in the skin. This is supported by reports in the literature, describing an increase in IL-1RA, CXCL-1/2 and hBD-1, and a decrease in IL-1α, in psoriasis lesional skin in comparison with non-lesional skin, or skin or healthy individuals have been reported in the literature. Thus, it appears that non-invasive measurements of soluble proteins found on the skin, e.g. as measured by FibroTx TAP, both qualitatively and quantitatively correlate with proteins found in the skin, as measured by invasive methods such as immunohistochemistry and qPCR from skin biopsies [38; 40; 42–45].

The decrease in pro-inflammatory IL-1α and increase in anti-inflammatory IL-1RA levels detected on psoriasis plaques in comparison with non-lesional skin, may appear counter-intuitive at first. Also, using a skin-lavage technique, Portugal-Cohen et al found a clear increase in IL-1α on lesional skin in comparison with non-lesional skin, or skin of healthy individuals [23]. In the literature, however, there is ample evidence that IL-1α is found in decreased levels, and IL-1RA in increased levels in psoriatic lesional skin in comparison with non-lesional skin [39, 42, 43]. FibroTx TAP measurements of IL-1α and IL-1RA thus fit the bulk of evidence in the literature. The reason for the discrepancy between FibroTx TAP measurements and the observations of Portugal-Cohen is unclear, also because using a similar skin-lavage approach as Portugal-Cohen, we found the same pattern for IL-1α and IL-1RA as we found using FibroTx TAP [34].

Despite the very clear association between disease and expression patterns of IL-1α, IL-1RA, CXCL-1/2 and hBD-1, as evidenced by the statistically significant differences in expression of these proteins on non-lesional and lesional skin, no firm correlations could be established between IL-1α, IL-1RA, hBD-1or CXCL-1/2 and PASI scores of the patients in the present study. A simple conclusion is that measurements of analysed biomarkers on a single lesion, or the ratio between IL-1RA and IL-1α, may not be representative for ‘whole body’ diagnostic purposes. This, however, is contradicted by our observation that clinical scoring of a single lesion, either for redness or thickness, significantly correlated with PASI in our study. Rather, the lack of correlation between measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 and PASI may be explained by the lack of significant correlations with clinical assessment of redness, thickness and scaling of the same lesions, which are elements that comprise the PASI in addition to scoring other lesions and body-surface area affected by disease. Nevertheless, the patient cohort of current study was limited, and a study with larger cohort of patients is needed for firm conclusions.

Ultrasound measurements clearly showed a statistically significant thickening of epidermis, SLEB and dermis in lesional skin in comparison with non-lesional skin. Despite a similar trend for FibroTx TAP measurements of IL-1α, IL-1RA and hBD-1, no clear quantitative correlations could be found between protein measurements and ultrasound measurements of epidermis, SLEB or dermis, neither with respect to thickness nor to quality of individual skin layers. At least not with the small number of patients used. Mild positive correlation between CXCL-1/2 and SLEB thickness of lesional skin was observed. Interestingly, neither ultrasound measurements and visual assessments of lesional skin correlated in a highly statistically significant sense; only a mild correlation between skin thickness and SLEB thickness was observed, and thus it appears that visual -, ultrasound – and protein-measurements quantify disease intensity in their own sense.

To address if measurements of skin-surface IL-1α, IL-1RA, CXCL-1/2 and hBD-1 merely reflect disease in a qualitative state, i.e. inflamed or not-inflamed, or that these measurements reflect disease-intensity quantitatively, we followed patients during the course of short-wave UVB treatment. There were clear patterns of normalisation observed for IL-1RA and CXCL-1/2. This pattern was gradually, thus confirming that skin-surface measurements of these proteins can be used to assess psoriasis-intensity in a qualitative way. Skin-surface measurements of IL-1RA and CXCL-1/2 displayed a different pattern than achieved by visual scoring of local inflammation. Visual scores for redness, thickness and scaling

decreased after 2 weeks of treatment, whereas IL-1RA and CXCL-1/2 normalized more gradually. This confirms that measuring the ‘molecular root’ of inflammation appears to have value as an objective, non-invasive biomarker measurement for scoring disease intensity on its own right. The difference in kinetics between IL-1α, unchanged during treatment, IL-1RA and CXCL-1/2, both changed albeit with different kinetics, suggest that changes in skin-surface proteins are not a uniform reflection of skin-healing, but rather reflect individual changes of expression in the skin.

# Conclusions

In conclusion, using FibroTx TAP we could measure clear differences in amounts of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on skin from psoriasis patients, with clear differences between lesional and non-lesional skin. Correlating FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on skin from psoriasis patients with clinical assessments of psoriasis severity, suggest that these protein measurements may have potential as biomarkers. Nonetheless, a substantially larger study will be necessary to further validate measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on skin from psoriasis patients for diagnostic, prognostic and therapeutic biomarker applications.

No adverse events were reported in FibroTx TAP measurements, neither on normal appearing skin nor on lesional skin, neither in patients nor in healthy individuals were reported, neither by visual assessment (e.g. signs of redness) or upon inquiry (e.g. irritation, itching, pain).

# Abbreviations

BSA body surface area

hBD-1 human antimicrobial beta defensin 1

ELISA enzyme-linked immunosorbent assay

IHC immuno-histochemistry

IL- α interleukin one alpha

IL-1RA interleukin one receptor antagonist

IL-6 interleukin six

IL-12 interleukin twelve

IL-23 interleukin twenty-three

hBD-1 human beta defensin 1

CXCL-1 chemokine (C-X-C motif) ligand 1

CXCL-2 chemokine (C-X-C motif) ligand 1

CXCL–1/2 chemokine (C-X-C motif) ligand 1/2

K16 keratin sixteen

PASI psoriasis area severity index

SCORAD SCOring Atopic Dermatitis severity index

SLEB sub-epidermal low echogenic band

TAP Transdermal Analysis Patch

TNF- α tumor necrosis factor alpha

UVB ultraviolet B

qPCR quantitative polymerase change reaction

# Declarations

### Ethics approval and consent to participate

Ethical approval for the studies is covered by Decision No. 2551 from the Tallinn Medical Research Ethical Committee. Prior the study, the detailed aim of the study was explained to each of the volunteer and an informed consent to participate was signed voluntarily by each of patient.

### Consent for publication

All authors have read and approved the final manuscript.

### Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and additional files.

### Competing Interests

Non to declare.

### Funding

Current research was funded by FibroTx LLC and by the Estonian Research Council as personal research Grant PUT1465 to KK.

### Authors contribution

KO and PS wrote the manuscript. KK, KA, MK, KO and KS conducted TAP biomarker measurements from the skin of psoriasis patients. KO, KS, JA, designed and performed experiments related to TAP biomarker measurement performed on of skin of healthy volunteers. KA and AM performed ultrasound measurements. TN and PS were responsible for the overall study design.

**Acknowledgement**

Not applicable

# References

1. Ayala F. Clinical presentation of Psoriasis. Reumatismo (2007); 59 Suppl 1:40 – 5.
2. Schön MP, et al. Psoriasis: Clinical manifestations, pathogenesis and therapeutic perspectives. Discov Med. 2005;5(27):253–8. Jun.
3. Yosipovitch G, et al. Impact of ixekizumab treatment on itch and Psoriasis Area and Severity Index in patients with moderate-to-severe plaque psoriasis: an integrated analysis of two phase III randomized studies. Dermatology therapy. 2018;8(4):621–37.
4. Langley RGB, et al. Psoriasis: epidemiology, clinical features, and quality of life. Ann Rheum Dis. 2005;64:18–23.
5. Bata-Csörgö Z, Szell M. The Psoriatic Keratinocytes. Expert Rev Dermatol. 2012;7(5):473–81.
6. Schön MP, Henning Boehncke W, Psoriasis. N Engl J Med. 2005;352:1899–912.
7. Prey S, et al. Assessment of risk of psoriatic arthritis in patients with plaque psoriasis: a systematic review of the literature. J Eur Acad Dermatol Venereol. (2010). Suppl 2:31 – 5.
8. Ashcroft DM, et al. Clinical measures of disease severity and outcome in psoriasis: a critical appraisal of their quality. Br J Dermatol. 1999;141(2):185–91.
9. Schmitt J, Wozel G. The psoriasis area and severity index is the adequate criterion to define severity in chronic plaque-type psoriasis. Dermatology. 2005;210(3):194–9.
10. Carlin CS, Feldman SR, Krueger JG, Menter A, Krueger GG. A 50% reduction in the Psoriasis Area and Severity Index (PASI 50) is a clinically significant endpoint in the assessment of psoriasis. J Am Acad Dermatol. 2004;50(6):859–66.
11. Unholzer A. KortingH.High-frequency ultrasound in the evaluation of pharmacological effects on the skin. Skin PharmacolAppl Skin Physiol. 2002;15(2):71–84.
12. Cucoş M, Crişan M, Lenghel M, Dudea M, Croitoru R, Dudea SM. Conventional ultrasonography and sonoelastography in the assessment of plaque psoriasis under topical corticosteroid treatment - work in progress. Med Ultrason. 2014;16(2):107–13.
13. Nguyen T, et al. Practice of phototherapy in the treatment of moderate-to-severe psoriasis. Curr Probl Dermatol. 2009;38:59–78.
14. Șomlea M, Cristina, et al. High-frequency ultrasonography of psoriatic skin: A non-invasive technique in the evaluation of the entire skin of patients with psoriasis: A pilot study. Experimental Therapeutic

Medicine. 2019;18(6):4981–6.
15. Kim IH, et al. Comparative efficacy of biologics in psoriasis: a review. Am J Clin Dermatol. (2012) Dec 1;13(6):365 – 74.
16. Laws PM, Young HS. Current and emerging systemic treatment strategies for psoriasis. Drugs. 2012 Oct 1;72(14):1867-80.
17. Rustin MH. Long-term safety of biologics in the treatment of moderate-to-severe plaque psoriasis: review of current data. Br J Dermatol. 2012;167(Suppl 3):3–11. Nov.
18. Patel RV, et al. Treatments for psoriasis and the risk of malignancy. J Am Acad Dermatol. 2009;60(6):1001–17. Jun.
19. Biomarkers Definitions Working Group. et al. "Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology therapeutics. 2001;69(3):89–95.
20. Gottlieb, et al. TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. J Immunol. 2005;175(4):2721–9.
21. Portugal-Cohen, et al. Noninvasive skin measurements to monitor chronic renal failure pathogenesis. Biomed Pharmacother. 2011;65(4):280–5.
22. Portugal-Cohen M, Kohen R. Non-invasive evaluation of skin cytokines secretion: an innovative complementary method for monitoring skin disorders. Methods. 2013;61:63–8.
23. Portugal-Cohen, et al. Non-invasive skin biomarkers quantification of psoriasis and atopic dermatitis: cytokines, antioxidants and psoriatic skin auto-fluorescence. Biomed Pharmacother. 2012;66(4):293–9.
24. Malaviya R, et al. Induction of lesional and circulating leukocyte apoptosis by infliximab in a patient with moderate to severe psoriasis. J Drugs Dermatol. (2006) (9):890–3.
25. Dand N, et al. HLA-C* 06: 02 genotype is a predictive biomarker of biologic treatment response in psoriasis. Journal of Allergy Clinical Immunology. 2019;143(6):2120–30.
26. Gottlieb, et al. Infliximab in psoriasis. J Am Acad Dermatol. 2003;49:112–17.
27. Krueger JG, et al. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. J Exp Med. 1995;182(6):2057–68.
28. Lizzul PF, et al. Differential expression of phosphorylated NF-kappaB/RelA in normal and psoriatic epidermis and downregulation of NF-kappaB in response to treatment with etanercept. J Invest Dermatol. 2005;124(6):1275–83.
29. Lowes MA, et al. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci U S A. 2005;102(52):19057–62.
30. Cabrijan L, et al. Influence of PUVA and UVB radiation on expression of ICAM-1 and VCAM-1 molecules in psoriasis vulgaris. Coll Antropol. 2008;32(Suppl 2):53–6.

31. Usmani N, et al. Photochemotherapy for localized morphoea: effect on clinical and molecular markers. Clin Exp Dermatol. 2008;33(6):698–704.
32. Chodorowska G, et al. C-reactive protein and alpha2-macroglobulin plasma activity in medium-severe and severe psoriasis. J Eur Acad Dermatol Venereol. 2004;18(2):180–3.
33. Bukulmez, et al. Serum adenosine deaminase levels in patients with psoriasis: a prospective case-control study. Eur J Dermatol. 2000;10(4):274–6.
34. Orro K, et al. Development of TAP, a non-invasive test for qualitative and quantitative measurements of biomarkers from the skin surface. Biomark. Res, 2014.
35. Feldman SR, and Michael D. Zanolli. Phototherapy Treatment Protocols. CRC Press, 2016.
36. Kim B, et al. The interleukin-1α precursor is biologically active and is likely a key alarmin in the IL-1 family of cytokines. Frontiers in immunology. 2013;4:391.
37. Malyak M, et al. "Characterization of a low molecular weight isoform of IL-1 receptor antagonist.". J Immunol. 1998;161(4):1997–2003.
38. Šahmatova L, et al. Signs of innate immune activation and premature immunosenescence in psoriasis patients. Scientific reports. 2017;7(1):1–13.
39. Terui T, et al. An increased ratio of interleukin-1 receptor antagonist to interleukin-1α in inflammatory skin diseases. Exp Dermatol. 1998;7(6):327–34.
40. Falcone D, et al. Measurement of skin surface biomakers by transdermal analyses patch following different in vivo models of irritation: a pilot study. Skin Research Technology. 2017;23(3):336–45.
41. Chiricozzi A, et al. Integrative responses to IL-17 and TNF-α in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. Journal of Investigative Dermatology. 2011;131(3):677–87.
42. Janssens AS, et al. Reduced IL-1Ra/IL-1 ratio in ultraviolet B-exposed skin of patients with polymorphic light eruption. Exp Dermatol. 2009;18(3):212–7.
43. Tamilselvi E, et al. Association of Disease Severity with IL-1 levels in Methotrexate-treated Psoriasis Patients. Scand J Immunol. 2013;78(6):545–53.
44. Ozlu E, et al. The investigation of antimicrobial peptides expression and its related interaction with methotrexate treatment in patients with psoriasis vulgaris. Cutan Ocul Toxicol. 2017;36(4):321–6.
45. Uzuncakmak T, Kevser, et al. "Alteration of tissue expression of human beta defensin-1 and human beta defensin-2 in psoriasis vulgaris following phototherapy." Biotechnic & Histochemistry (2019): 1–6.

# Tables

Due to technical limitations, Tables 1-4 are provided in the Supplementary Files section.

CAPTIONS

**Table 1. Ratio of IL-1RA over IL-1a on the skin of healthy volunteers and on the skin of psoriasis patients** The average concentration of IL-1a and IL-1RA on normal skin of healthy volunteers (N = 10), non-lesional and lesional skin of psoriasis patients (N = 30) is presented in Table 1 in ng/ml. The standard deviation (SD) presented in table present the standard deviation from average of combined measurements in the 10 healthy volunteers and 30 psoriasis, respectively. Additionally, molar ratio of IL-1RA over precursor and mature IL-1a (Ratio of IL-1RA/IL-1a) is presented.

**Table 2. Analysed correlations of FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on lesional skin between PASI and local scores of thickness, scaling and redness in psoriasis patients.** Correlation between biomarker measurements and clinical scores of psoriasis patients (N = 30) was assessed using Spearman's rank correlation analysis. Statistical significances were verified with probability value (*p*-value). Relevant correlations are flagged with asterisk.

**Table 3A. Correlation analysis between FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on non-lesional skin of psoriasis patients combined with ultrasound measurements of epidermis-, dermis and SLEB- thickness at the same analysis site.** Correlation between biomarker measurements and skin layer thickness of psoriasis patients (N = 30) was assessed using Spearman's rank correlation analysis. Statistical significances were verified with probability value (*p*-value). Relevant correlations are flagged with asterisk. *$* p < 0.05$, $** p < 0.01$, $*** p < 0.001$.*

**Table 3B. Correlation analysis between FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on lesional skin of psoriasis patients and between ultrasound measurements of epidermis-, dermis and SLEB- thickness at the same analysis site.** Correlation between biomarker measurements and skin layer thickness of psoriasis patients (N = 30) was assessed using Spearman's rank correlation analysis. Statistical significances were verified with probability value (*p*-value). Relevant correlations are flagged with asterisk. *$* p < 0.05$, $** p < 0.01$, $*** p < 0.001$.*

**Table 3C. Correlation analysis between local scores and epidermal-, dermal and SLEB- thickness measured from lesional skin by ultrasound.** Correlation between local clinical scores and epidermal-, dermal and SLEB- thickness measured from lesional skin of psoriasis patients (N = 30) was assessed using Spearman's rank correlation analysis. Statistical significances were verified with probability value (*p*-value). Relevant correlations are flagged with asterisk. The FibroTx TAP measurements, clinical scores and ultrasound measurements were performed all at the exact same skin lesion. *$* p < 0.05$, $** p < 0.01$, $*** p < 0.001$*

**Table 4 Ratios between IL-1RA and IL-1a on non-lesional and lesional skin of psoriasis patients.** The average concentration of IL-1a and IL-1RA on non-lesional (NL) and lesional (L) skin of psoriasis patients (N = 14) is presented in Table 4 in ng/ml. The standard deviation (SD) in table presents the standard deviation from average of combined measurements in psoriasis patient NL and L skin site, respectively. Additionally, molar ratio of IL-1RA over precursor and mature IL-1a (Ratio of IL-1RA/IL-1a) is presented.

# Figures

**Figure 1A.**

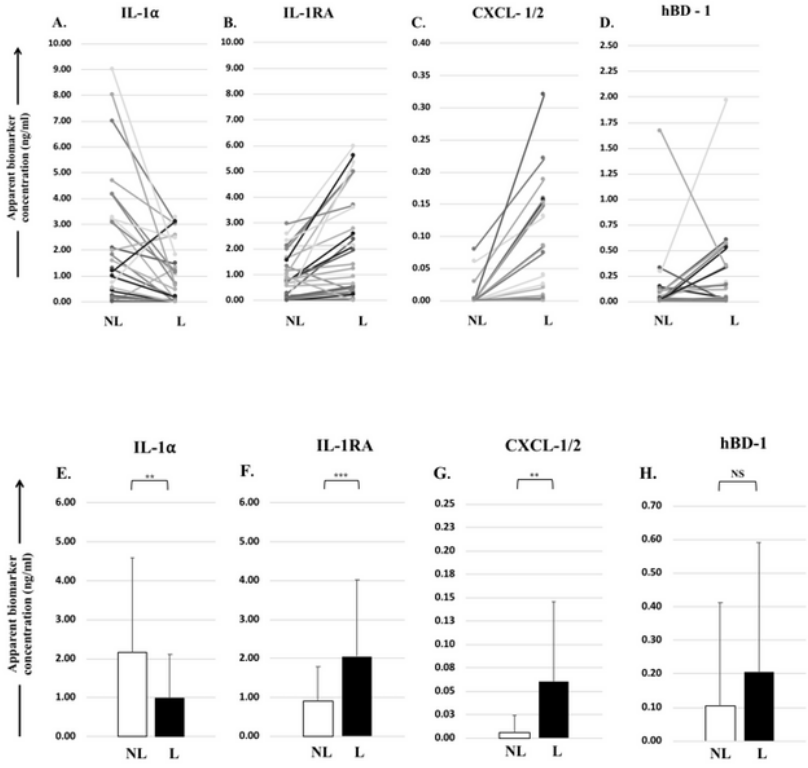



**Figure 1B.**

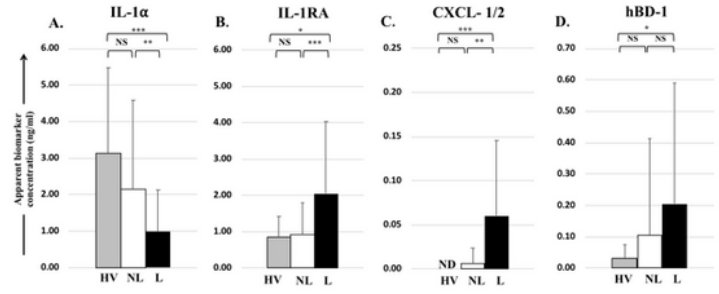
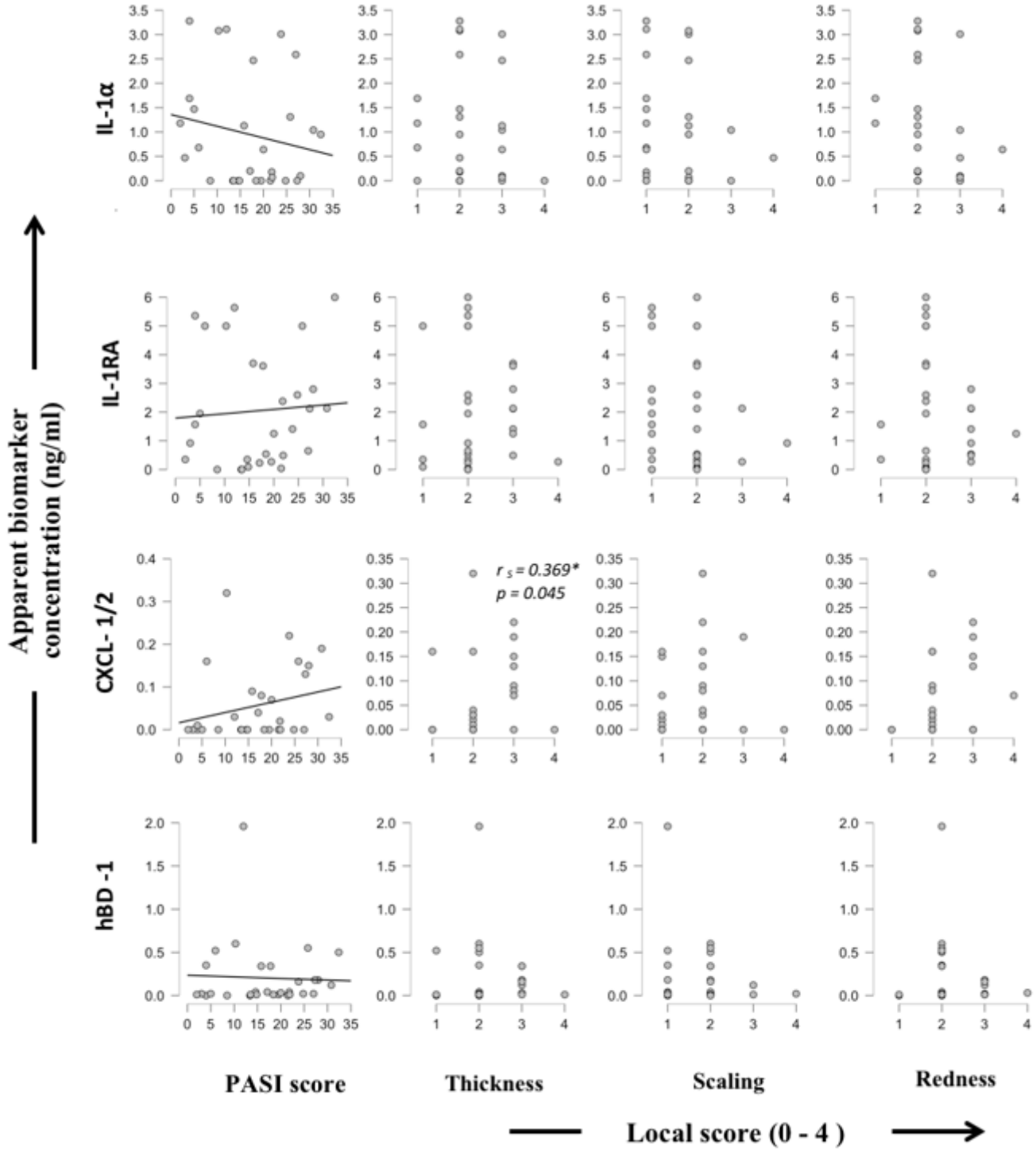



Figure 1

A. Measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on non-lesional and lesional skin of psoriasis patients using FibroTx TAP In panel A-D single measurements of IL-1αIL-1RA, CXCL-1/2 and hBD-1detected from non-lesional (NL) skin and lesional skin (N) of 30 psoriasis patients have been depicted, each line represents a single patient. In panel E-H the apparent average biomarker concentrations of IL-1αIL-1RA, CXCL-1/2 and hBD-1detected from non-lesional skin (white bars) and lesional skin (black bars) have been blotted. Y-axis: Apparent concentration of analysed biomarker on skin in ng/ml. X-axis: sampling site. Error bars on graphs present the standard deviations for average of combined measurements in the participants. Statistical significance (paired sample t-test) is indicated on panel E-H: *p < 0.05, ** p < 0.01, *** p < 0.001; NS- not significant. B. Measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on lesional and non-lesional skin of psoriasis patients and normal skin of healthy volunteers using FibroTx TAP. The apparent average biomarker concentrations of IL-1αIL-1RA, CXCL-1/2 and hBD-1 detected from the skin surface of 10 healthy volunteers (N=10; grey bars), on non-lesional skin (NL; white bars) and lesional skin (L; black bars) of 30 psoriasis patients have been blotted. Y-axis: Apparent concentration of analysed biomarker on skin in ng/ml. X-axis: sampling site. Error bars on graphs present the standard deviations for average of combined measurements in the participants. Statistical significance (student t-test) is indicated on panel E-H: *p < 0.05, ** p < 0.01, *** p < 0.001; ND – not detected; NS - not significant.




## Figure 2

Analysed correlations of FibroTx TAP measurements of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on lesional skin between PASI and local scores of thickness, scaling and redness on the lesional skin of psoriasis patients. Correlation between biomarker measurements and clinical scores of psoriasis patients (N = 30) was assessed using Spearman's rank correlation analysis. Statistical significances were verified with probability value (p-value, presented in Table 2). Y-axis: apparent concentration of detected biomarker in

ng/ml. X-axis: measurements of PASI score, local inflammation score of thickness, scaling and redness, respectively, on psoriasis lesion under analysis.

**Figure 3A.**

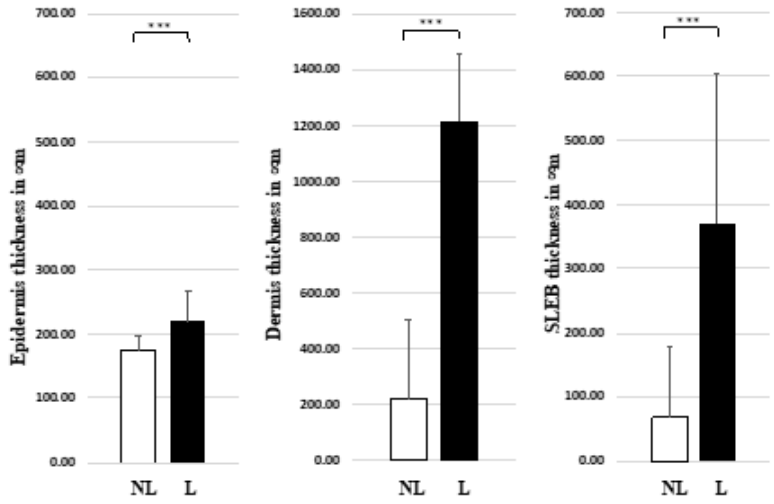
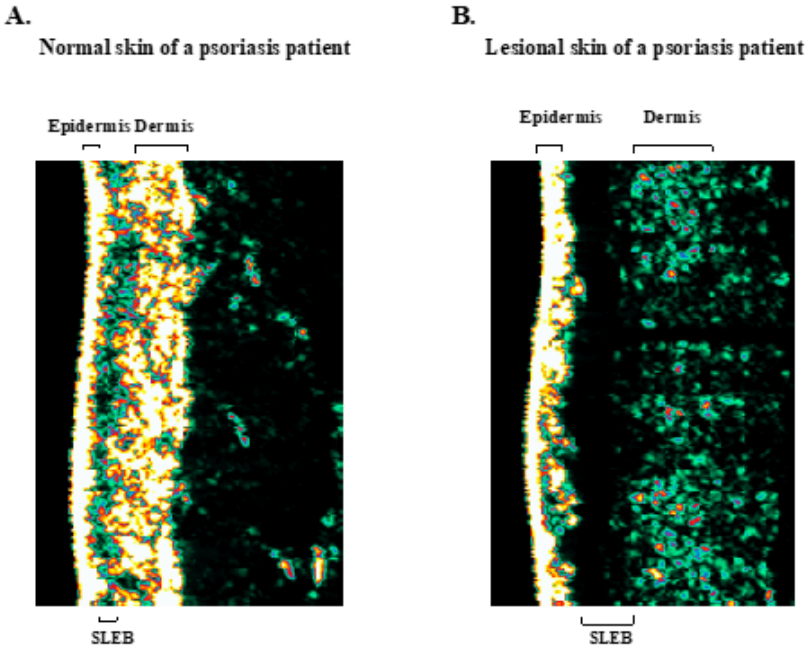



**Figure 3B.**




## Figure 3

Thickness of epidermis, dermis and SLEB in non-lesional and lesional skin of psoriasis patients measured by ultrasound. The apparent average epidermal thickness (panel A), dermal thickness (panel B) and SLEB thickness (panel C) analysed from non-lesional skin (NL; white bars) and lesional skin (L; black

bars) have been blotted. Y-axis: Average thickness of epidermis, dermis and SLEB, respectively, in µm. X-axis: sampling site. Error bars on graphs present the standard deviations for average of combined measurements in the participants. Statistical significance (paired sample t-test) is indicated on panel, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Figure 4A.**

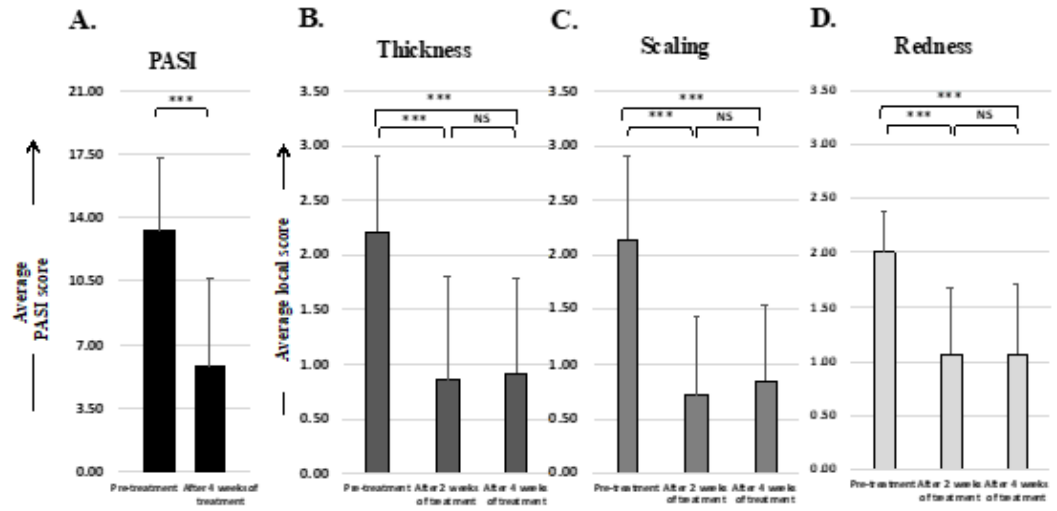



**Figure 4B.**

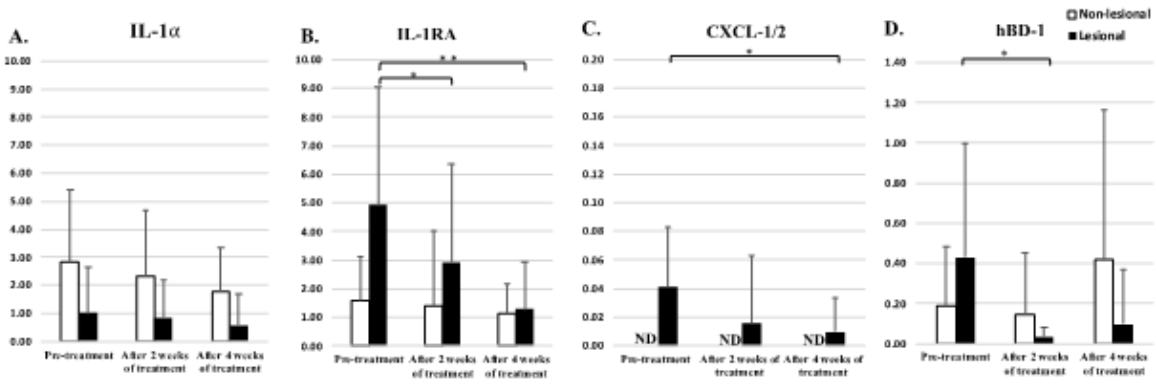



**Figure 4**

A Changes in the psoriasis area severity index (PASI) and local inflammation scores induced by narrow-band UVB treatment. The PASI score was documented before the treatment initiation and after four weeks of treatment (panel A). Local inflammation scores thickness (panel B), scaling (panel C) and redness (panel D) were documented before the treatment initiation, after two weeks and four weeks of treatment at the exact same lesions. Each bar plotted on Figure 4A panel A-D represents an average measurement of analysed clinical score of psoriasis patients (N=14). Error bars on graphs present the standard deviations for average of combined measurements in patients. Statistical significance was determined with paired sample Student t-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The paired sample student t-test values for local inflammation score comparison is presented in supplementary data, Table 2. B Measurements of IL-1, IL-1RA, CXCL-1/2 and hBD-1on healthy apparent and lesional skin of psoriasis patients during narrow band UVB treatment. Biomarker measurements were performed on healthy apparent and lesional skin before, after two and four weeks of treatment at the exact same skin site using FibroTx TAP. The apparent average biomarker concentration of non-lesional skin are plotted with white bars (panel A - D), average biomarker measurements of lesional skin site are presented with back bars (panel A – D). Y-axis: apparent concentration of IL-1α, IL-1RA, CXCL-1/2 and hBD-1 on skin in ng/ml. X-axis: time point of biomarker sampling. Error bars in graph A-D represent the standard deviations for average of combined measurements in patients (N = 14); ND- not detected. Statistical significance was determined with paired sample Student t-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), all the Student t-test p-values for are presented in supplementary data, Table 1.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pptx
- Tables.pptx