Clinical Implications"

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1. Introduction

Biomarkers are defined broadly as "any substance, structure, or process that can be measured in the body or its products and that influences or predicts the incidence of a result or disease" [1]. More specifically, biomarkers collected from the skin surface can be related to skin barrier function, inflammation, oxidative status, skin microbiome, etc. They include cytokines, chemokines, Anti-Microbial Peptides (AMP), components of Natural Moisturization Factor (NMF), keratinocyte differentiation proteins, oxidative stress markers (lipid and protein peroxidation), microbiome metabolites, etc.

Sampling the skin surface for biomarkers can be performed non-invasively using adhesive tapes, cotton swabs [2], transdermal patches [3, 4], or skin surface washes [5]. Following its extraction, the choice of biochemical analysis method depends on the specific biomarker being studied, the biological context, and the desired sensitivity and specificity of detection. Such methods include Enzyme-Linked Immunosorbent Assay (ELISA), Liquid Chromatography, Mass Spectrometry, Western Blotting, protein oxidation assays (e.g. DNPH), Polymerase Chain Reaction (PCR), Next-Generation Sequencing (NGS), etc.

Interleukin (IL)-1α and IL-1 Receptor Antagonist (IL1RA) are important biomarkers in regulating inflammation and maintaining skin homeostasis. Increased levels of IL-1α can signal compromised barrier function and heightened inflammatory responses. IL1RA helps balance inflammatory responses and supports the restoration of barrier function after injury or inflammation. Altered levels of IL1RA can indicate an imbalance in the inflammatory process. The concentration ratio of IL1RA/IL-1α is considered as a non-specific marker of skin inflammation [6]. Human Beta-Defensins (hBD) is a family of AMP relating to the skin's innate immune response and its barrier function [7]. hBD3, specifically, is distinguished by its potent antimicrobial activity against a broad spectrum of pathogens, including gram-positive and gram-negative bacteria, and its ability to retain activity under physiological salt conditions [8].

Despite their importance, challenges in skin surface biomarker assessment include variability in marker expression, limitations of sampling techniques, small volume, and standardization issues which can lead to inconsistencies across studies. In this study we test the feasibility of measuring non-invasively small changes in skin surface biomarkers (IL-1, IL1RA, and hBD3) following daily application of cosmetic products over a period of 7 days.

2. Materials and Methods

2.1. Volunteers and Study Design

A clinical study was conducted at our labs in Hamburg, Germany to obtain skin swab samples from test sites that had been treated with different products (curd soap, a marketed moisturizing soothing cream and concentrated lemon juice). Ten healthy male and female human volunteers (mean age 51.1 ± 17.5 years, range 20.8 - 64.9) with Fitzpatrick phototype I - III were recruited after having given written informed consent prior to participation. The study was conducted in accordance with the principles of the Declaration of Helsinki. Before the study begun, the volunteers were asked to refrain from any leave-on products on their forearms for a period of 7 days. Following this period, four test areas (5x5 cm) were marked on the forearms randomly assigned to receive the following treatments: a) untreated control, b) curd soap, c) a marketed moisturizing soothing cream, d) concentrated lemon juice. The products were applied twice daily to the assigned test sites over 7 days. Skin evaluations were performed before the first product application (baseline) and one day after the last application. All measurements were performed in a climate-controlled room at 21.5°C (± 1°C) and 50 % (±5%) relative humidity after the subjects had adapted with their uncovered arms to these indoor climate conditions for at least 30 min.

2.2. Instrumental Evaluation and Skin Surface Sampling

To evaluate the barrier function, trans epidermal water loss (TEWL) was measured using an open chamber device (DermaLab, Cortex Technology, Denmark). The predefined measurement sequence was over a period of 45 seconds. The mean of the last measured eight values of this sequence was recorded. Skin colour was measured using a spectrophotometer (CM-700d, Konica Minolta, Japan) in the L*a*b* system as defined by the Commission Internationale de l'Eclairage (CIE).

At skin evaluation, swab samples were also collected after tape stripping (two tapes). At the control site a swab was also collected after 5 tape strips. For swab sample collection, test sites were rinsed with swabs (FLOQ Swabs, Hain Lifesience GmbH, Germany) using 2 ml of a 1% Tween in PBS rinsing buffer. After centrifugation, the supernatants were stored at -80°C for further analysis. The swab samples were used to evaluate the amounts of total protein (BCA Assay, Immundiagnostik, Germany), hBD3 (Human BD-3 Super X-ELISA, Antigenix America, USA), IL1α (Human IL1α ELISA assay, Arigo Biolaboratories, Taiwan) and IL1RA (Human IL1RA ELISA assay, Arigo Biolaboratories, Taiwan).

2.3. Data Analysis

Statistical analyses were performed using a custom code written in Python and the Jamovi software v.2.3 [9]. For comparisons between two groups (baseline vs one day after treatment and 2 vs 5 tapes for the untreated site), first the Shapiro-Wilk test was used to assess normality of the data distributions. Then, the Student's T-test was used if both groups were normally distributed, otherwise the Wilcoxon rank test. The percent change from the baseline value was also calculated as an indicator of biological relevance. Correlations between parameters were evaluated using the the Pearson's coefficient. For all tests the significance level was set at a=0.05.

3. Results

3.1. Skin Barrier Function

Of the tested treatments only the skin sites that were treated with curd soap showed a significant and biologically relevant increase in TEWL rates (Figure 1), indicating that one week

of twice daily washing with curd soap is enough to cause measurable change in the skin barrier function.

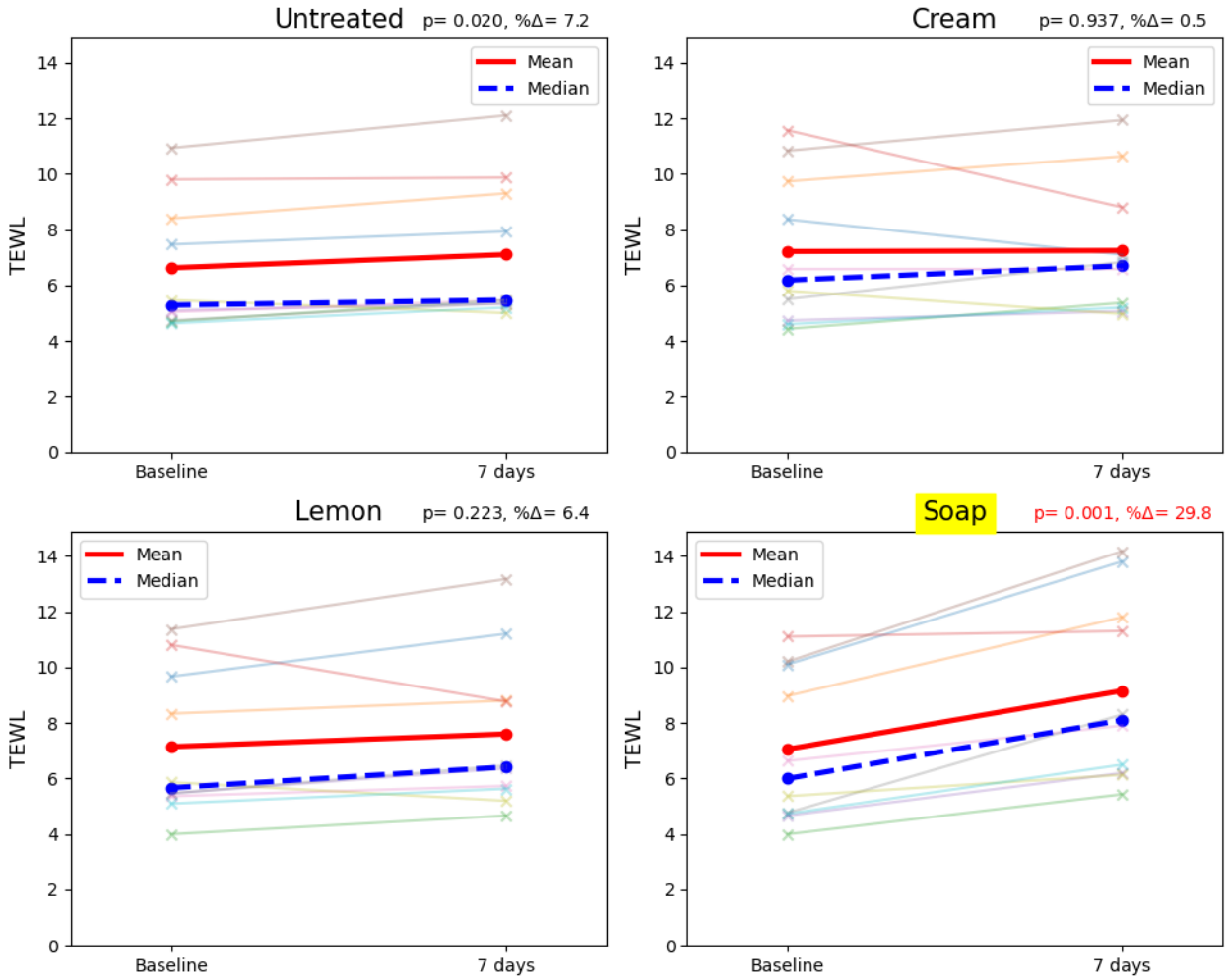

Figure 1. Change in trans-epidermal water loss rates (TEWL) between baseline and 7 days for the different treatment conditions. %Δ indicates mean per cent change from baseline. Highlighted are the treatments for which both $p<0.05$ and $\%\Delta > 10$. TEWL is given in $g \cdot m^{-2} \cdot h^{-1}$.

3.2. Erythema

There was no significant change in the values of a* (green-to-red aspect of skin color) between baseline and 7 days for any treatments, indicating the absence of any visible erythema due to treatment.

3.3. Inflammatory Markers

Of the tested treatments only the skin sites that were treated with curd soap showed a significant and biologically relevant increase in the skin surface concentrations of IL-1a (Figure 2), indicating that the damage in skin barrier function caused by one week of twice daily washing with curd soap was accompanied with an increase in inflammation.

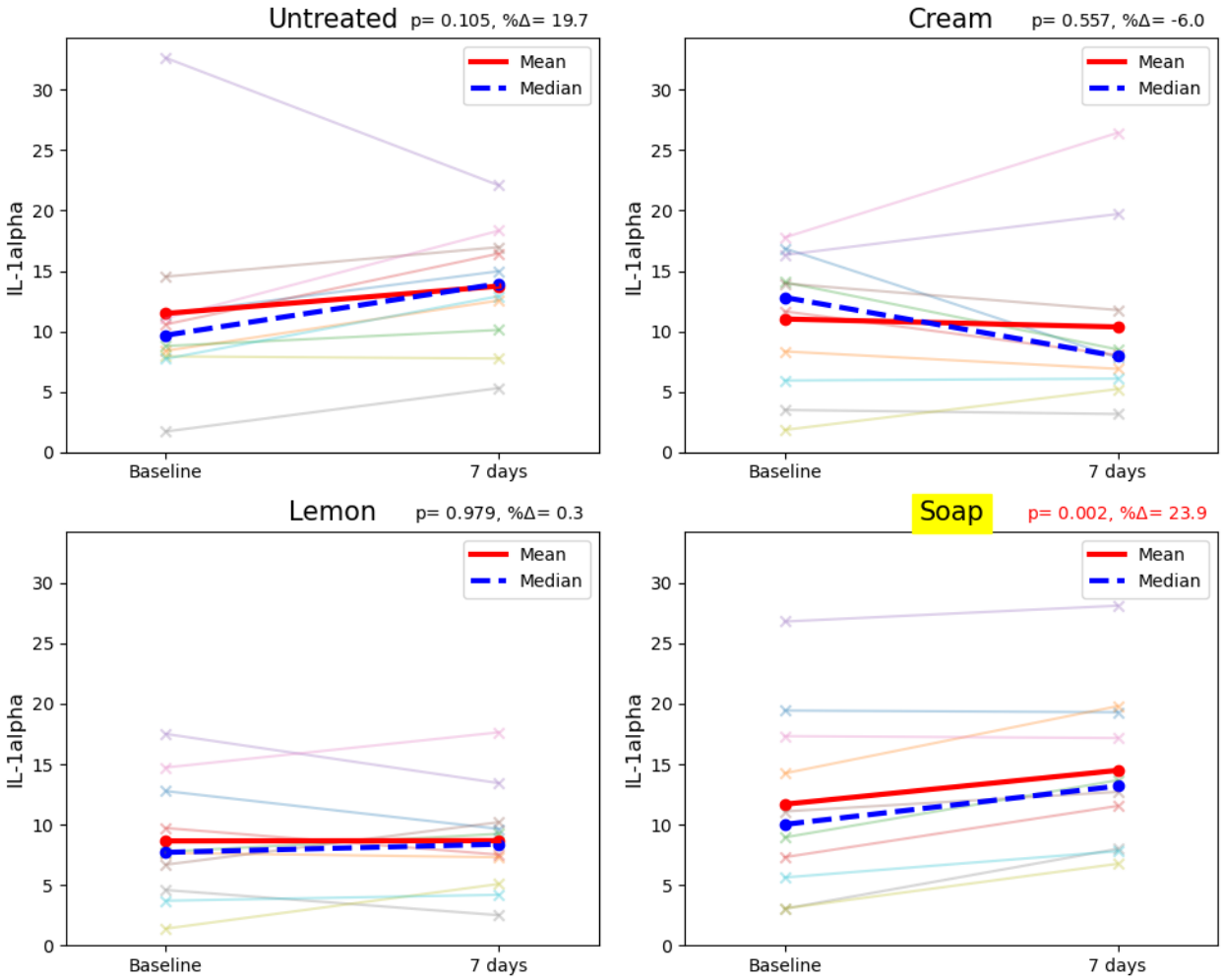

Figure 2. Change in the skin surface concentration of IL-1α between baseline and 7 days for the different treatment conditions. %Δ indicates mean per cent change from baseline. Highlighted are the treatments for which both $p<0.05$ and %Δ > 10. The concentration is given in picograms per microgram to total protein.

Both treatment with lemon juice and the soothing moisturizing cream product demonstrated a significant decrease in skin surface concentration of IL1RA (Figure 3) and a significant reduction in the concentration ratio of IL1RA/IL-1α (Figure 4), both indicating the soothing action of the cream and the effect of supporting the skin acid mantle.

3.4. Anti-microbial Marker

The soothing effect of the cream was evidenced also by a significant reduction in the skin surface concentration values of hBD3. However, the untreated site also showed a significant decrease which was attributed to a high variability in the baseline values. Therefore, for this marker it is recommended to use a larger number of volunteers in a study.

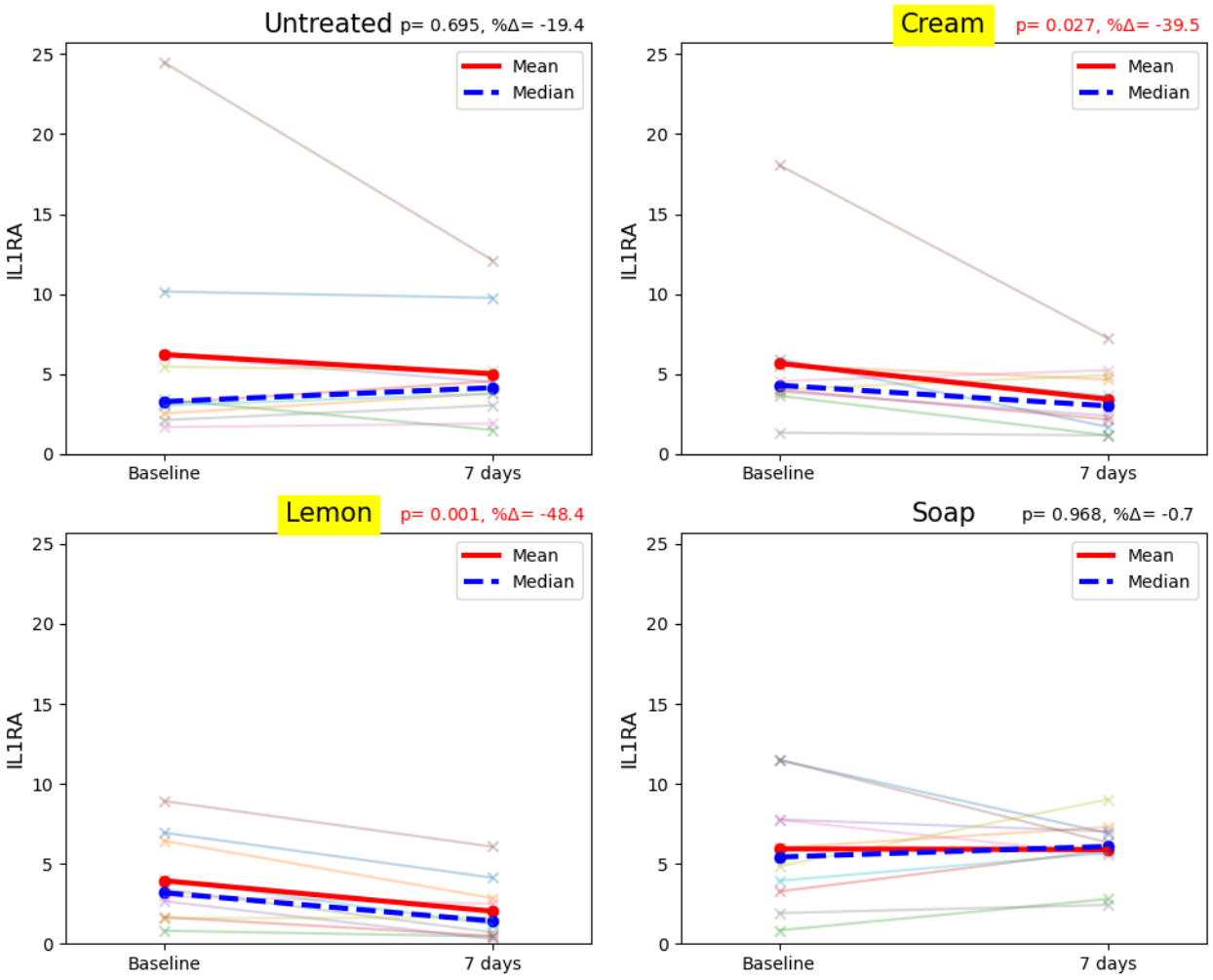

Figure 3. Change in the skin surface concentration of IL1RA between baseline and 7 days for the different treatment conditions. %Δ indicates mean per cent change from baseline. Highlighted are the treatments for which both $p<0.05$ and %Δ > 10. The concentration is given in picograms per microgram to total protein.

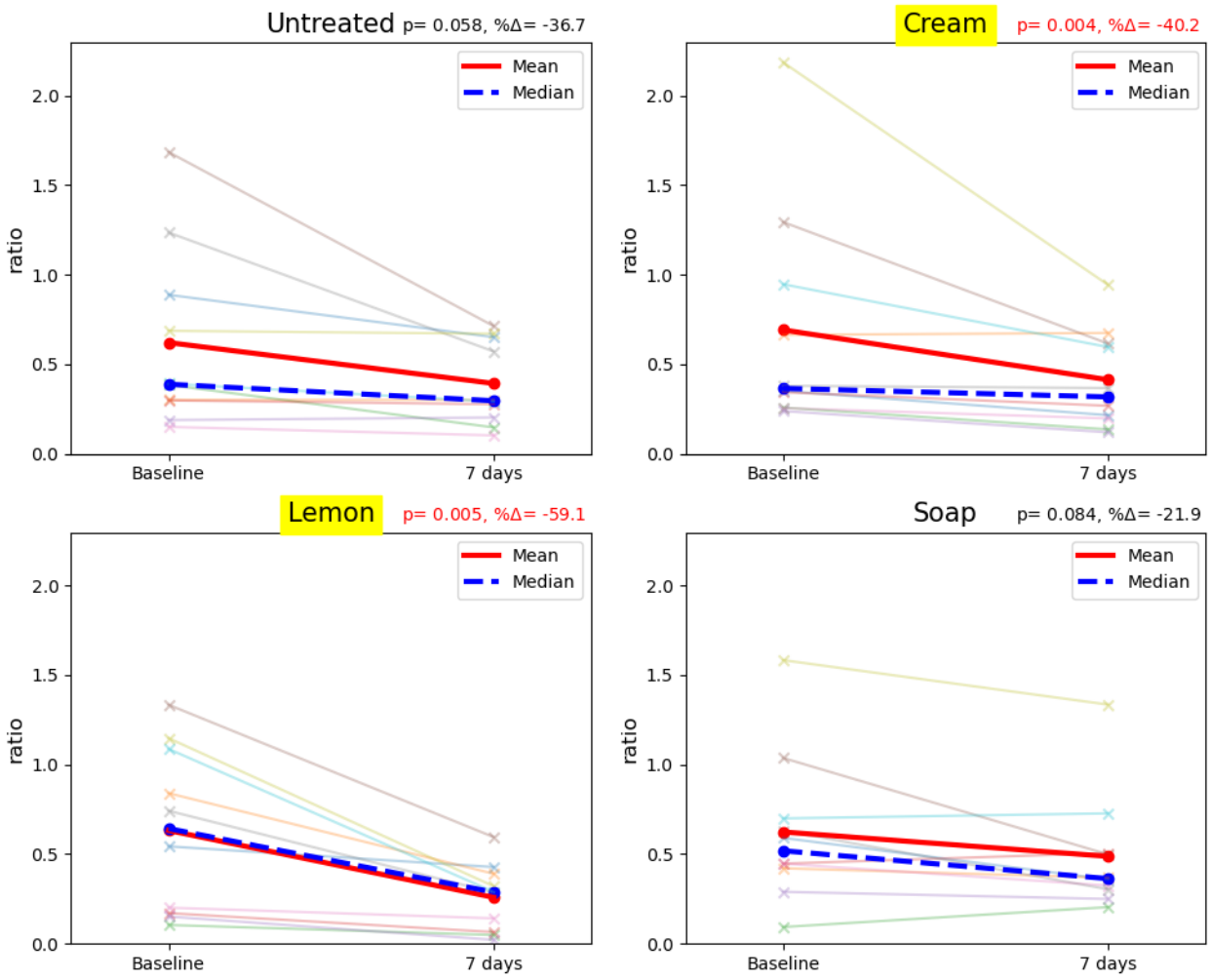

Figure 4. Change in the ratio of the skin surface concentrations IL1RA/IL-1α between baseline and 7 days for the different treatment conditions. %Δ indicates mean per cent change from baseline. Highlighted are the treatments for which both $p<0.05$ and $\%\Delta > 10$.

3.5. Depth profiles

On the untreated sites samples were collected following 2 and 5 tape strips to explore the effect of depth dependance of the measured biomarker concentrations. Indeed, the concentrations of IL-1a and IL1RA increased as a function of depth, indicating that these molecules are transferred through diffusion to the skin surface (Figure 5). Notably the ratio did not change significantly as a function of depth. The concentration of hBD3 also did not change significantly which could indicate a faster diffusion compared to the interleukins due to its smaller size (~5 kDa for hBD3 vs. ~17-20 kDa for IL-1α and IL1RA).

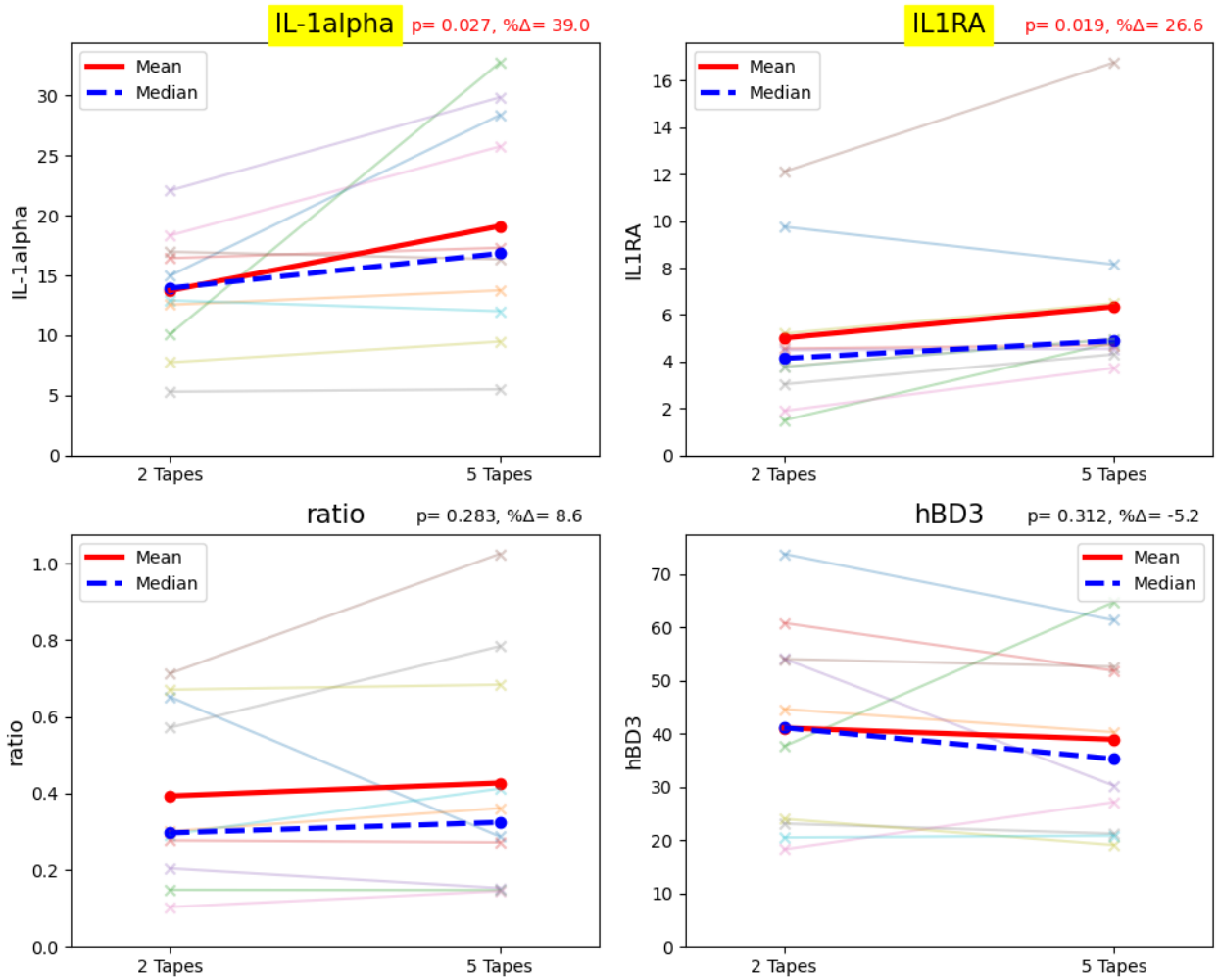

Figure 5. Change in the ratio of the skin surface concentrations of the measured biomarkers as a function of depth in the Stratum Corneum following removal of 2 and 5 tapes. %Δ indicates mean per cent change from baseline. Highlighted are the treatments for which both $p<0.05$ and $\%\Delta > 10$.

3.5. Multiparametric Analysis

A cross-correlation matrix for all parameters measured shows several moderate but significant correlations several pairs of them (Figure 6), indicating that skin barrier and inflammation pathways are interconnected. Similar values of the correlation coefficients were found for the measurement following 7 days of treatment.

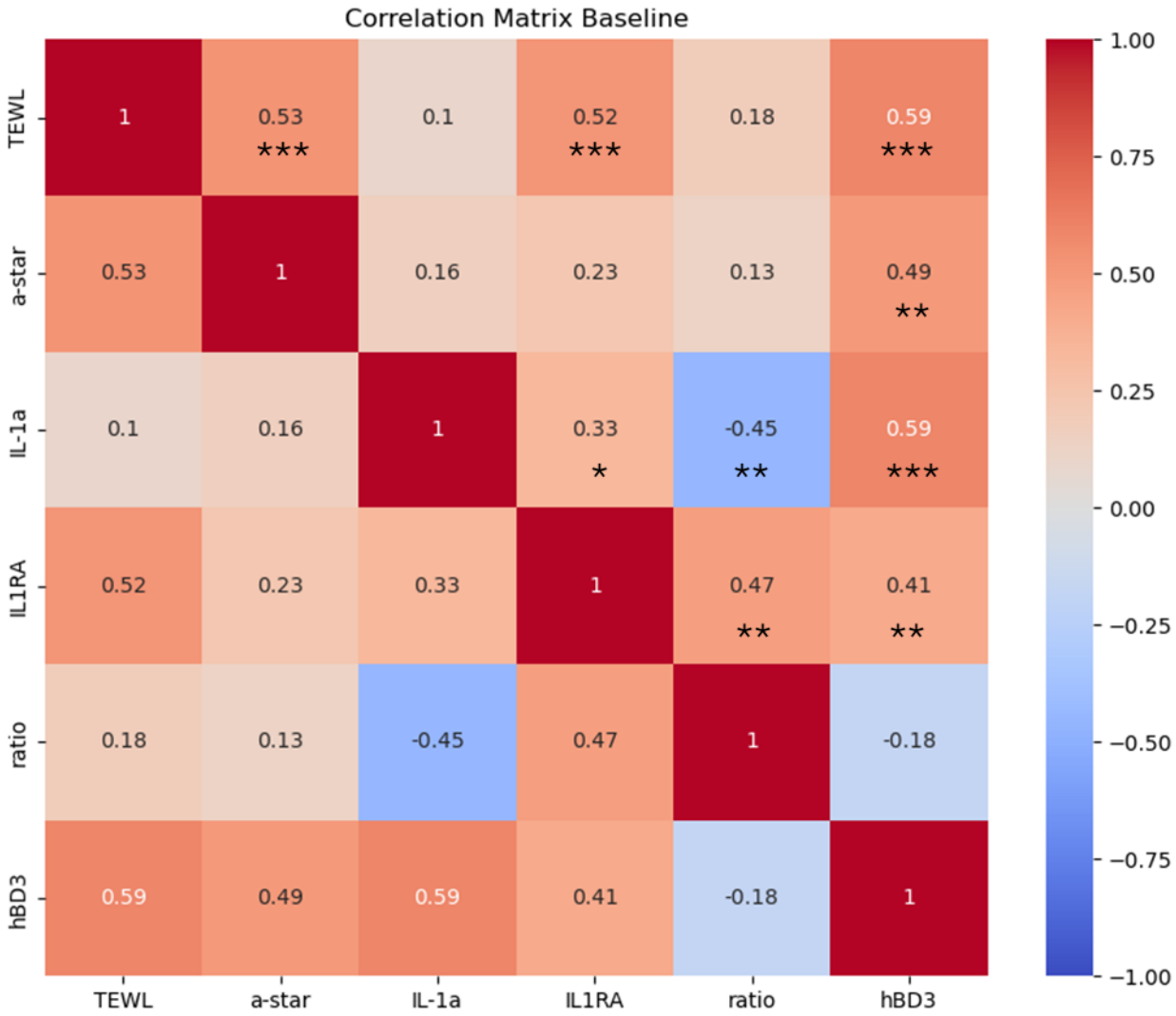

Figure 6. Change in the ratio of the skin surface concentrations of the measured biomarkers as a function of depth in the Stratum Corneum following removal of 2 and 5 tapes. %Δ indicates mean per cent change from baseline. * p<0.05; ** p<0.01; *** p<0.001.

4. Discussion

In this study we demonstrated the feasibility of measuring small changes in skin surface biomarkers following a week-long application of topical products.

Our results show that daily use of curd soap causes a measurable damage to the skin barrier as measured by TEWL without any clinical indication of erythema. This barrier damage is accompanied by a measurable increase in the skin surface concentration of IL-1α.

Lemon juice was used as a low pH buffer. The observation that it decreased the concentration of IL1RA and thereby the ratio IL1RA/IL-1α, may be indicative of the beneficial effect of topical products formulated to maintain the skin surface pH close to the physiological acidic values (pH 5-5.5).

The benefits of a topical soothing cream were demonstrated by the reduction of the IL1RA/IL-1α concentration ratio and the skin surface concentration of HBD3.

Skin surface biomarkers have emerged as a promising approach to study subclinical changes in the physiologic state of the skin. They can provide valuable insights, enabling researchers to assess various aspects of skin-product interaction. Measuring skin surface biomarkers non-invasively is important in the assessment of topical product safety and tolerability as well as documenting product efficacy in skin soothing. Mechanistic studies can also benefit from measurements of skin surface biomarkers by elucidating the biological pathways involved in skin responses to products. Furthermore, biomarkers can reveal new benefit areas for cosmetic products, such as enhancing skin barrier function or reducing oxidative stress. Lastly, the use of biomarkers facilitates personalization in skincare by enabling tailored product recommendations based on individual skin profiles, thereby enhancing treatment outcomes and consumer satisfaction. Thus, skin surface biomarkers are valuable in advancing both cosmetic science and personalized dermatology.

5. Conclusion

The ability to analyze skin surface biomarkers facilitates early detection of diseases, enhances treatment customization, and improves patient outcomes. Consequently, this innovative approach is poised to revolutionize clinical practices in dermatology, promoting a more holistic understanding of skin-related health issues.

6. References

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