

# Challenges in Noninvasive Skin Biomarker Measurements in Daily Practice: A Longitudinal Study on Skin Surface Protein Detection by the Transdermal Analysis Patch in Pediatric Psoriasis

Mirjam J. Schaap<sup>a</sup> Finola M. Bruins<sup>a</sup> Noa J.M. van den Brink<sup>a</sup> Kadri Orro<sup>b,c</sup>  
Hans M.M. Groenewoud<sup>d</sup> Elke M.G.J. de Jong<sup>a</sup> Ellen H. van den Bogaard<sup>a</sup>  
Marieke M.B. Seyger<sup>a</sup>

<sup>a</sup>Department of Dermatology, Radboud University Medical Center, Nijmegen, The Netherlands; <sup>b</sup>FibroTx LLC, Tallinn, Estonia; <sup>c</sup>Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia; <sup>d</sup>Department for Health Evidence, Radboud University, Nijmegen, The Netherlands

## Keywords

Cytokines · Stratum corneum · Children · Longitudinal study · Tape stripping · Marker

## Abstract

**Introduction:** Skin surface proteins are potential biomarkers in psoriasis and can be measured noninvasively with the transdermal analysis patch (TAP). This study aimed to assess markers measured by TAP over time in daily clinical practice, explore their correlation with disease severity in pediatric psoriasis, and compare the TAP and tape stripping detection capability. **Methods:** In this prospective observational daily clinical practice study, pediatric psoriasis patients (aged >5 to <18 years) were followed during 1 year. At each visit, TAPs were applied to lesional ( $n = 2$ ), peri-lesional ( $n = 2$ ), and non-lesional ( $n = 1$ ) sites. Post-lesional skin was sampled if all lesions on the arms, legs, or trunk cleared. Treatment and psoriasis severity data were collected. IL-1RA, hBD-2, IL-1 $\alpha$ , IL-8, VEGF, CXCL-1/2, CCL-27, IL-23, hBD-1, IL-22, IL-17A, KLK-5, and IL-4 levels were quantified by spot-ELISA. For the statistical analysis, Wilcoxon signed rank tests, Mann-Whitney U tests, and Spearman correlations were used. Detection capa-

bility of the TAP was compared to tape stripping in a separate cohort of adult psoriasis patients. **Results:** 32 patients (median age 15.0 years, median Psoriasis Area and Severity Index [PASI] 5.2) were followed for a mean of 11.3 ( $\pm 3.4$ ) months with a total of 104 visits. In lesional skin ( $n = 197$ ), significantly higher IL-1RA, hBD-2, IL-8, VEGF, CXCL-1/2, IL-23, hBD-1, IL-22, CCL-27, and IL-17A levels were found compared to non-lesional skin ( $n = 104$ ), while IL-1 $\alpha$  was higher in non-lesional skin. Marker levels were highly variable over time and did not correlate with disease severity measured by PASI or SUM scores. Comparison of the TAP and tape strip detection capability in adult psoriasis patients ( $n = 10$ ) showed that lesional hBD-2, IL-1 $\alpha$ , IL-8, and VEGF and non-lesional IL-1RA, hBD-2, IL-8, and VEGF were more frequently detected in tape extracts than TAPs. **Conclusion:** Due to the lack of correlation with clinical disease severity and the current detection capability of the markers measured by TAP in psoriasis, its use in regular practice is still a bridge too far.

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Ellen H. van den Bogaard and Marieke M.B. Seyger contributed equally to this work.

## Introduction

Psoriasis is a common chronic inflammatory skin disease with an onset during childhood in almost one-third of all cases [1]. Its disease course is characterized by exacerbations and remissions and is unpredictable: some patients have a mild and stable disease for many years, while in other patients, psoriasis might progress quickly [2]. Our understanding of the pathophysiology of (plaque) psoriasis and involved cytokines (e.g., IL-17, IL-23, TNF- $\alpha$ ) has rapidly increased over the past decades [3, 4]. If biomarkers were able to predict disease course or response to treatment, their added value to daily practice would be considerable, especially in facilitating early intervention and improving treatment decisions. The skin surface provides a unique source of potential biomarkers as cytokines, chemokines, growth factors, and antimicrobial peptides can be measured directly at the site of the disease and are thought to reflect the underlying pathophysiology [5–8].

Proteins in psoriatic skin are conventionally sampled through skin biopsies [9–12], which are associated with a risk of pain, scarring, and infection [13]. Noninvasive biomarker measurements provide a patient-friendly alternative by avoiding discomfort and fear for interventions, especially in pediatric patients, and can be performed repeatedly, making them more suitable for use in daily clinical practice. Tape stripping, during which corneocytes of the superficial stratum corneum are sampled with adhesive tape, is regarded as the golden standard for noninvasive skin protein sampling and is widely performed [7, 14, 15]. Previous tape stripping studies have underlined the value and power of local biomarkers [7, 15]. In psoriasis and/or atopic dermatitis, numerous proteins were successfully detected in the stratum corneum using tape stripping, such as interleukins (e.g., IL-1 $\alpha$ , IL-1 $\beta$ ) or chemokines (e.g., CXCL-1, CXCL-8) [7, 15, 16]. Recently, the transdermal analysis patch (TAP) and the FibroTx patch were described [17–19]. These methods sample soluble proteins from an intact stratum corneum. Previous research by our group revealed that the TAP can detect skin proteins in lesional, peri-lesional, and non-lesional skin in pediatric psoriasis patients treated with systemic and/or topical agents and is regarded as patient friendly [17, 19].

The consistency of proteins measured by TAP over time and the correlation with disease severity are not studied to date but are relevant to identify potential biomarkers for use in clinical practice. Additionally, the skin surface protein detection capability of the TAP might dif-

fer from that of tape stripping since these methods sample at different depths of the stratum corneum and include different detection methods. The primary objective of this study, performed in a daily clinical practice setting, was to explore the value of TAP measurements in daily practice in (pediatric) psoriasis patients by (i) comparing the marker levels measured by TAP in lesional, peri-lesional, non-lesional, and post-lesional skin, (ii) assessing the course of skin surface markers measured by TAP over time in pediatric psoriasis patients, and (iii) exploring the correlation between lesional marker levels and disease severity. In addition, the marker detection capability of TAP was compared to tape stripping extraction in a pilot study in adults with psoriasis.

## Materials and Methods

### *Study Design and Population*

In this prospective observational daily clinical practice study, pediatric and adolescent psoriasis patients aged 5–18 years were recruited between June 2018 and July 2019 at the outpatient clinic of the Department of Dermatology of the Radboud University Medical Center, Nijmegen, The Netherlands. Inclusion criteria were a dermatologist-confirmed plaque psoriasis diagnosis and sufficient psoriasis plaques to apply the TAPs at baseline. Patients with another concurrent inflammatory skin disease or other types of psoriasis were excluded. Treatment occurred as part of regular care without a washout phase and could consist of topical and/or systemic treatment. Patients were followed during 1 year, and visits were planned every 3 months for systemically treated patients and every 6 months for solely topically treated patients. However, no visits occurred between March and August 2020 due to COVID-19 restrictions. Written informed consent was given by all participants aged  $\geq 16$  years. Participants aged 12–16 years gave written informed assent. Legal guardians of participants aged under 16 years gave written informed consent before enrollment. The study was approved by the Ethics Committee of the Radboud University Medical Center, region Arnhem-Nijmegen (NL60952.091.17).

### *Transdermal Analysis Patch*

The TAP is developed and commercialized by FibroTx and consists of a multiplex capture-antibody microarray supported by an adhesive bandage. The TAP method is described in the online supplementary Materials (for all online suppl. material, see [www.karger.com/doi/10.1159/000527258](http://www.karger.com/doi/10.1159/000527258)) and previous publications [17, 19]. Two TAPs were used with different preset protein panels. One TAP measured CXC chemokine ligand (CXCL)-1/2, interleukin (IL)-1RA, IL-23, IL-1 $\alpha$ , IL-8, vascular endothelial growth factor (VEGF), and human beta-defensin (hBD)-2, and a second TAP measured CC chemokine ligand (CCL)-27, IL-4, IL-22, IL-17A, hBD-1, and kallikrein-related peptidase (KLK)-5. After application, four drops of phosphate-buffered saline (pH 7.4) were added to microarray reservoir. TAPs were applied to the skin for 20 min to capture skin-derived proteins through immune recognition and were stored at  $-20^{\circ}\text{C}$  until quantification with spot-enzyme-linked immunosorbent assay (spot-ELISA). Levels within half of the low-

er detection limit were taken unchanged, lower values were substituted by zero. The TAP detection limits are provided in online supplementary Table S1.

#### Study Procedures

TAPs were applied to two lesional, two peri-lesional, and one non-lesional skin sites (no psoriasis within a distance of 10 cm). The two lesions of interest were preferably located on different body parts, including the arms, legs, or trunk, with preferably a similar SUM score. TAPs were applied to the same skin sites at each visit during follow-up if possible. However, if all lesions on the arms, legs, or trunk cleared, TAPs were applied to post-lesional skin instead of lesional skin. Patients did not use topical agents on the investigated sites on the day of the visit.

After TAP removal, pediatric patients rated experienced discomfort on a simplified 10-point visual analogue scale (VAS). A VAS score of 0 and 10 corresponded to no and maximal discomfort, respectively. Psoriasis severity was measured with the Psoriasis Area and Severity Index (PASI; range 0–72) score, Physician Global Assessment (PGA; range 0–5), and affected Body Surface Area (BSA) [20–23]. SUM scores (0–12), defined as the sum of the severity scores for erythema (0–4), induration (0–4), and desquamation (0–4), were determined for the lesions of interest [24]. Additionally, demographics and information on current treatment were collected. All procedures were performed by two physicians (MJS or FMB).

#### TAP versus Tape Stripping

To compare the marker detection capability of TAP with tape stripping, we performed a pilot study in adult psoriasis patients from September to October 2020. Inclusion criteria were diagnosis of plaque psoriasis confirmed by a dermatologist, a plaque large enough to perform both TAP and tape stripping, and no other concurrent inflammatory skin disease. TAP and tape stripping were consecutively performed at one time point in a randomized order on one lesional and non-lesional skin site. Directly after each method, experienced discomfort was rated by the patient on a 100-mm VAS. TAP sampling and analysis were performed as previously described. For tape stripping, eight successive round adhesive tapes with a diameter of 2.2 cm (DSquame, CuDerm, USA) were pressed to the skin for 10 s using a pressure device (CuDerm, USA). Tapes were stored in cryovials at  $-80^{\circ}\text{C}$  until analysis. Proteins were extracted from the tapes using ultrasonication with PBS and were either quantified by conventional ELISA (hBD-2) or Luminex multiplex-based platform (CXCL-1/2, IL-1RA IL-1 $\alpha$ , IL-8, and VEGF) and normalized to the total amount of protein. Values exceeding the detection limits were not included in the analysis. Detailed methods are provided in the online supplementary Material. All patients gave written informed consent. The study was approved by the Ethics Committee of the Radboud University Medical Center, region Arnhem-Nijmegen (NL73363.091.20).

#### Statistical Analysis

Patient characteristics and marker levels were first analyzed with descriptive statistics and presented as frequencies and percentages, means and standard deviations ( $\pm$ SD), or medians and interquartile ranges (IQRs). Marker levels on both the two lesional and peri-lesional sites within 1 patient were compared on group level with a Wilcoxon signed rank test. Mann-Whitney U tests were performed to compare marker levels in lesional, peri-lesional, non-lesional, and post-lesional skin. Mean lesional marker lev-

**Table 1.** Baseline characteristics

Characteristic	(N = 32) <sup>a</sup>
Sex, male, n (%)	17 (53.1)
Age, years, median (IQR <sup>b</sup> )	15.0 (10.9–16.9)
Psoriasis severity, median (IQR <sup>b</sup> )	
PASI <sup>c</sup>	5.2 (3.7–8.7)
PGA <sup>d</sup>	2.0 (2.0–3.8)
BSA <sup>e</sup>	6.9 (4.6–10.8)
SUM score, <sup>f</sup> median (IQR <sup>b</sup> )	5.0 (4.0–6.0)
Site location, n (%)	
Lesional <sup>f</sup>	
Leg	25 (39.1)
Arm	24 (37.5)
Trunk	15 (23.4)
Non-lesional	
Leg	19 (59.4)
Arm	9 (28.1)
Trunk	4 (12.5)
Solely topical treatment, n (%)	19 (59.4)
Systemic treatment, <sup>g</sup> n (%)	
Methotrexate	9 (28.1)
Fumaric acid esters	1 (3.1)
Adalimumab	3 (9.4)
Total	13 (40.6)
Switched from the therapy group during follow-up, n (%)	
From solely topical to systemic treatment	4 (12.5)
From systemic to solely topical treatment	2 (6.3)

BSA, body surface area; PGA, Physician Global Assessment. <sup>a</sup>Unless stated otherwise. <sup>b</sup>The 25th and 75th percentile are shown. <sup>c</sup>Psoriasis Area and Severity Index score (range 0–72). <sup>d</sup>Physician Global Assessment (range 0–5). <sup>e</sup>Body surface area (%). <sup>f</sup>All lesions of interest combined, resulting in a total of 64 lesions (2 lesions per patient) at baseline. <sup>g</sup>Patients may also use topical treatments (corticosteroids and/or vitamin D derivatives).

els and the PASI score were plotted over time in each patient. Spearman rank correlation tests were computed for the PASI score and mean lesional marker levels and for lesion severity (total SUM score, desquamation, erythema, induration) and lesional marker levels. The TAP and tape stripping detection capability was assessed by comparing the number of samples that were detected within the limits of the assay for each marker. Statistical package SPSS, version 25 (IBM, Armonk, NY) and SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) were used to perform analyses. A two-sided  $p < 0.05$  was regarded statistically significant.

## Results

### Patient Characteristics

Thirty-two patients were included with a median age of 15.0 years (IQR 10.9–16.9) and a median PASI score of 5.2 (IQR 3.7–8.7) at baseline (Table 1). A mean of 3.25

**Table 2.** Marker concentrations measured in lesional, peri-lesional, non-lesional, and post-lesional skin samples

Marker	Lesional (n = 197), n (%)	Peri-lesional (n = 197), n (%)	Non-lesional (n = 104), n (%)	Post-lesional (n = 6), n (%)	p value <sup>a</sup>	p value <sup>b</sup>	p value <sup>c</sup>	p value <sup>d</sup>	p value <sup>e</sup>	p value <sup>f</sup>
IL-1RA	3.65 (3.33)	1.68 (2.10)	1.14 (1.73)	5.11 (2.70)	<0.001	0.009	<0.001	<0.001	0.002	0.178
hBD-2	2.84 (2.99)	1.21 (2.25)	0.51 (1.28)	7.63 (6.98)	<0.001	0.001	<0.001	<0.001	0.001	0.022
IL-1 $\alpha$	0.92 (1.31)	1.56 (1.81)	1.89 (2.18)	1.28 (1.18)	<0.001	0.123	<0.001	0.683	0.927	0.171
IL-8	0.52 (0.97)	0.03 (0.10)	0.01 (0.11)	0.09 (0.15)	<0.001	0.001	<0.001	0.002	0.150	0.135
VEGF	0.23 (0.43)	0.06 (0.19)	0.01 (0.06)	0.21 (0.33)	<0.001	0.028	<0.001	<0.001	0.023	0.714
CXCL-1/2	0.08 (0.50)	0.04 (0.35)	0.04 (0.35)	0.01 (0.02)	<0.001	0.163	<0.001	0.511	0.826	0.156
CCL-27	0.04 (0.09)	0.01 (0.02)	0.01 (0.02)	0.00 (0.01)	<0.001	0.058	<0.001	0.796	0.699	0.173
IL-23	2.30 (2.64)	1.52 (2.09)	1.56 (2.71)	2.86 (2.90)	0.002	0.381	0.002	0.213	0.266	0.609
hBD-1	0.22 (0.28)	0.15 (0.21)	0.13 (0.18)	0.17 (0.14)	0.042	0.195	0.004	0.245	0.392	0.851
IL-22	0.10 (0.25)	0.04 (0.12)	0.01 (0.04)	0.06 (0.09)	0.092	0.098	0.004	0.137	0.393	0.740
IL-17A	0.03 (0.13)	0.01 (0.07)	0.00 (0.01)	0.03 (0.06)	0.203	0.248	0.049	0.005	0.084	0.295
KLK-5	29.49 (49.98)	25.16 (29.01)	24.92 (31.40)	33.48 (7.26)	0.875	0.707	0.814	0.028	0.041	0.045
IL-4	0.00 (0.05)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.156	1.000	0.302	1.000	1.000	0.804

All visits were included in this analysis. Values are presented as means (standard deviation) in ng/mL, and comparisons were performed with Mann-Whitney U tests. Means instead of medians are displayed to improve the interpretation of the results. <sup>a</sup> Lesional versus peri-lesional skin. <sup>b</sup> Peri-lesional versus non-lesional skin. <sup>c</sup> Lesional versus non-lesional skin. <sup>d</sup> Post-lesional versus non-lesional skin. <sup>e</sup> Post-lesional versus peri-lesional skin. <sup>f</sup> Post-lesional versus lesional skin.

( $\pm 1.0$ ) visits were performed per patient, resulting in a total of 104 visits and a mean follow-up duration of 11.3 ( $\pm 3.4$ ) months. Due to COVID-19 restrictions, 10 patients dropped out before 1 year of follow-up, longer visit intervals occurred toward the end of follow-up, and follow-up exceeded 1 year in 14 patients. Of the 10 patients that dropped out early, still two to four visits were performed and data of these visits were included in the analysis. At baseline, 19 patients received solely topical treatment, while 13 patients received systemic treatment. During follow-up, 4 patients switched from topical to systemic treatment, while 2 patients switched from systemic to solely topical treatment (Table 1). Mild adverse events of TAP were reported during six visits, including transient local erythema ( $n = 2$ ), itch ( $n = 3$ ) and a burning sensation ( $n = 1$ ). The median VAS score after TAP removal was 1.0 (IQR 0.0–1.0).

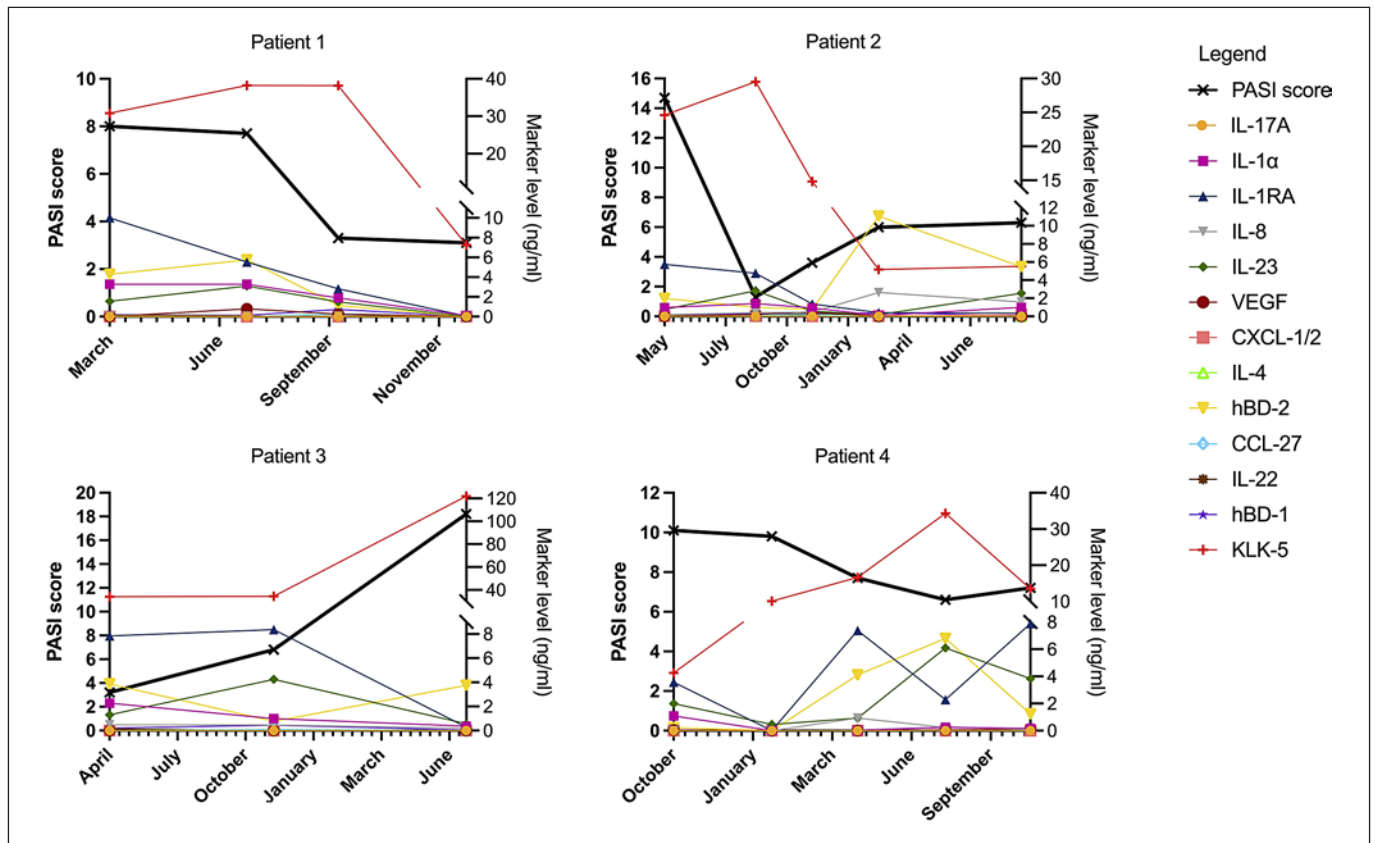
#### Marker Level Differences between Lesional, Peri-Lesional, Non-Lesional, and Post-Lesional Skin

When including all samples on both the two lesional and two peri-lesional sites, marker levels were considered similar (online suppl. Table S2). Therefore, all data were taken together for further analysis, resulting in 197 lesional, 197 peri-lesional, 104 non-lesional, and 6 post-lesional TAP measurements. The TAP was able to distinguish lesional from non-lesional skin: significantly higher levels of IL-1RA, hBD-2, IL-8, VEGF, CXCL-1/2, CCL-27, IL-23, hBD-1, and IL-17A were found in lesional skin, whereas higher levels of IL-1 $\alpha$  were found in non-lesional skin (Table 2). In peri-lesional skin, IL-1RA, hBD-2, IL-8, and VEGF levels remained significantly higher compared to non-lesional skin. IL-4 was only (and barely) detected in two lesional samples and did not show any significant relations. In addition, if all lesions on the arms, legs, or trunk cleared during follow-up, TAPs were applied to post-lesional skin (online suppl. Fig. S1). Intriguingly, post-lesional marker levels were equal to lesional levels or even significantly higher (for hBD-2 and KLK-5, Table 2). Moreover, compared to non-lesional skin, levels of hBD-2, IL-8, VEGF, IL-17A, and KLK-5 were significantly higher in post-lesional skin. A post hoc analysis solely including the patients in which post-lesional skin was sampled during follow-up revealed similar trends.

#### Lesional Marker Levels over Time and Their Correlation with Disease Severity

Detected lesional marker levels highly varied between analyzed skin surface proteins (Table 2). To explore the course of marker levels during follow-up in each patient,





**Fig. 1.** PASI scores and lesional marker levels measured by TAP over time. PASI scores (depicted on the left y axis) and mean lesional marker levels (depicted on the right y axis) are visualized over time for four representative patients. The follow-up duration is plotted on the x axis with intervals of 3 months. Marker levels are shown in ng/mL.

mean lesional marker levels were plotted over time. PASI scores were added to these figures to explore if the trend of marker levels followed the overall disease severity. Regarding the association between marker concentrations and disease severity (PASI score) over time, inter- and intra-patient differences were seen. Four representative patients are depicted in Figure 1. To further explore this association, correlations between marker levels and the PASI score were calculated (Table 3). These correlations were weak ( $<0.30$ ) and not statistically significant (except for KLK-5). Mostly inverse correlations were obtained, indicating lower marker levels for higher PASI scores (Table 3). Furthermore, the association between marker concentrations and lesion severity scores (SUM scores) was assessed. Analysis showed mostly inverse correlations between desquamation severity and marker levels, reaching statistical significance for IL-1 $\alpha$ , hBD-1, and KLK-5 (Table 3). The possible association between lower marker levels and more desquamation was further sub-

stantiated by calculating mean marker levels for each local desquamation score. As the amount of lesional desquamation increased, a clear trend of decreasing IL-1 $\alpha$ , VEGF, CCL-27, IL-23, hBD-1, IL-22, IL-17A, and KLK-5 levels was observed (online suppl. Table S3). This trend was less distinct for the local erythema and induration scores (data not shown). Of note, only for IL-8, a weak positive correlation was found with lesion severity scores ( $<0.3$ ;  $p < 0.01$ , Table 3).

#### *TAP versus Tape Stripping Detection Capability*

To compare the detection capability of TAP with tape stripping, an additional study was performed including noninvasive protein profiling of stratum corneum by both TAP and tapes. Both methods were performed on the same lesion and non-lesional skin site in 10 adult psoriasis patients (median age 58.0, median PASI 2.0) at one time point. Most patients were treated with systemic agents ( $n = 7$ ; 70.0%). Full population characteristics are

**Table 3.** Correlation between lesional marker concentrations and disease severity

Marker	Lesion severity <sup>a</sup> (N = 197)								Overall disease severity (N = 99): PASI score <sup>b</sup>	
	total SUM score		desquamation		erythema		induration		correlation coefficient	p value
	correlation coefficient	p value	correlation coefficient	p value	correlation coefficient	p value	correlation coefficient	p value		
IL-1RA	-0.070	0.326	-0.032	0.653	-0.037	0.607	-0.120	0.092	0.094	0.353
hBD-2	-0.026	0.718	-0.133	0.063	-0.025	0.731	0.060	0.400	-0.140	0.163
IL-1 $\alpha$	-0.244	0.002	-0.239	0.001	-0.171	0.016	-0.155	0.030	-0.190	0.058
IL-8	0.217	0.002	0.204	0.004	0.201	0.005	0.103	0.149	0.117	0.247
VEGF	-0.013	0.854	-0.075	0.296	0.117	0.100	-0.097	0.176	-0.113	0.265
CXCL-1/2	0.070	0.331	0.069	0.336	0.078	0.274	0.029	0.683	-0.051	0.616
CCL-27	-0.069	0.339	-0.129	0.071	0.000	0.999	-0.055	0.444	-0.111	0.274
IL-23	-0.101	0.158	-0.125	0.081	-0.077	0.282	-0.059	0.407	-0.003	0.974
hBD-1	-0.012	0.870	-0.146	0.041	0.079	0.270	0.018	0.806	-0.113	0.266
IL-22	-0.050	0.485	-0.013	0.855	-0.050	0.487	-0.046	0.518	-0.173	0.088
IL-17A	-0.017	0.810	-0.053	0.462	0.029	0.689	-0.008	0.909	-0.131	0.198
KLK-5	-0.111	0.120	-0.179	0.012	-0.026	0.714	-0.105	0.145	-0.211	0.036
IL-4	0.097	0.176	0.016	0.823	0.097	0.176	0.102	0.155	0.044	0.667

Spearman correlations are shown for lesion or disease severity and lesional marker concentrations. All lesional data were included in this analysis. No correction for multiple measures within 1 patient was performed. <sup>a</sup> For the correlation with lesion severity, the two lesional measurements at each patient visit were included separately and post-lesional samples were excluded, resulting in a total of 197 included measurements. Total SUM scores ranged from 2 to 9, desquamation scores ranged from 0 to 3, erythema scores ranged from 1 to 3, and induration scores ranged from 0 to 4. <sup>b</sup> For the correlation with the Psoriasis Area and Severity Index score, the mean lesional protein concentration was used for each patient visit. PASI scores ranged from 1.2 to 18.2.

shown in online supplementary Table S4. In general, markers were more often detected, or detected within the detection limits, in stratum corneum extracts from tape strip samples. Specifically, lesional hBD-2, IL1- $\alpha$ , IL-8, and VEGF and non-lesional IL-1RA, hBD-2, IL-8, and VEGF were more frequently detected in tape extracts (Table 4). Regarding the implementation of the sample techniques in daily practice, patients reported a low median VAS score for discomfort for both procedures: 0.0 mm (IQR 0.0–0.0 mm) for the TAP versus 0.00 (IQR 0.0–6.0 mm) for tape stripping. No adverse events were reported.

## Discussion

This study aimed to explore the value of TAP measurements in daily practice in (pediatric) psoriasis patients. We assessed skin surface proteins measured by TAP over time, determined their correlation with psoriasis severity, and compared the TAP detection capability with tape stripping. We showed that marker levels measured by TAP can differentiate between lesional and non-lesional skin. Surprisingly, marker levels in post-lesional skin were equal to or higher than lesional levels and signifi-

cantly higher than non-lesional levels. Lesional marker levels varied highly over time, and no convincing correlations were found between these marker levels and PASI or SUM scores. In addition, the protein detection capability by tape stripping appeared superior to the TAP for five out of six quantified markers in a separate cohort of 10 adults with psoriasis. In the next paragraphs, we will discuss the implication of these results.

In line with our previous publication on the baseline analysis of this cohort [19], IL-1RA, hBD-2, IL-8, VEGF, and CXCL-1/2 levels were significantly increased in lesional skin, while the level of IL-1 $\alpha$  was increased in non-lesional skin compared to lesional skin. In this follow-up analysis, IL-23, IL-22, hBD-1, CCL-27, and IL-17A were also significantly increased in lesional skin. These results are in line with a previous study by Mehul et al. [7] that used tape stripping for stratum corneum proteome profiling in adult psoriasis patients, which also found higher levels of VEGF, CXCL-1/2, IL-8, CCL-27, and IL-17A and lower levels of IL-1 $\alpha$  in lesional skin. In contrast, we found higher levels of IL-22 in the pediatric psoriatic stratum corneum, while this was not increased in adults in the study by Mehul et al. [7]. It has previously been suggested that IL-22 could differentiate pediatric from adult psoria-

sis [11]. As expected from the pathophysiology of psoriasis and previous studies [7, 19], IL-4 was barely detected: only in 2 out of 197 lesional samples.

In the pediatric cohort, a low correlation between TAP marker levels and disease severity was found. Since TAPs measured lower marker concentrations as the amount of lesional desquamation increased, excessive desquamation in psoriasis seems to hamper sampling of soluble skin surface proteins. Our results indicate that tape stripping has a greater protein detection capability and a greater fold change between non-lesional and lesional samples, which further substantiates potential detection issues using the TAP in psoriasis. The greater protein detection capability of tape stripping could be explained by sampling deeper layers of the stratum corneum, and a greater sensitivity of quantification methods used for the tape extracts (herein used Luminex platform) than the spot-ELISA used for the TAPs. As the pilot study in adults with psoriasis solely aimed to compare the detection capability of TAP and tape stripping, statements about the potential clinical value of markers derived from tape strips in psoriasis cannot be made. Further research is needed regarding the consistency of these marker levels over time and their correlation with disease severity. A second explanation for the low correlation between TAP marker levels and disease severity is the fact that the marker set captured with the TAP was a preset general inflammatory panel, thus not specific for psoriasis. Hence, other psoriasis-specific markers might yield better correlations with disease severity. Another hypothesis to explain the low correlation between TAP marker levels and disease severity might be that the marker levels detected on the stratum corneum surface reflect a delayed representation of the activity in the skin. In line with this hypothesis, measured post-lesional marker levels were equal to or higher than lesional levels. However, we postulate that actual lesional levels were even higher than post-lesional levels, but excessive desquamation resulted in an under-detection of lesional marker levels. Nonetheless, hBD-2, IL-8, VEGF, IL-17A, and KLK-5 levels were significantly higher in post-lesional compared to non-lesional skin. Multiple studies have revealed that molecular and cellular imprints of psoriasis do not fully disappear in clinically cleared skin [25–27]. However, given the small number of post-lesional samples ( $n = 6$ ), we are unable to make valid statements regarding the presence of a residual inflammatory scar, and results have to be confirmed in more post-lesional samples.

We note several limitations. Given the explorative study design, we did not perform correction for multiple

**Table 4.** Marker levels in lesional and non-lesional skin of adult psoriasis patients ( $N = 10$ ) measured by TAP and tape stripping

Marker	Lesional			Non-lesional			Detection capability comparison											
	TAP <sup>a</sup>			TAP <sup>a</sup>			tape stripping <sup>b</sup>			lesional			non-lesional					
	N <sup>c</sup>	median	range	N <sup>c</sup>	median	range	N <sup>c</sup>	median	range	both, n	only TAP, n	only tape strip, n	both, n	only TAP, n	only tape strip, n			
IL-1RA	10	7.339.41	3,150.50–10490.92	6 <sup>d</sup>	36.20	23.48–50.00	6	2413.52	256.50–3,835.23	7	2.00	0.22–4.91	10	-	-	5	1	2
hBD-2	8	3,149.33	800.73–4,574.33	10	295.04	20.88–767.56	0	-	-	5	1.81	0.02–10.72	8	-	2	-	-	5
IL-1 $\alpha$	6	319.12	222.91–896.29	10	0.28	0.00–0.84	10	1,656.17	169.12–9,017.55	10	12.84	3.55–71.86	6	-	4	10	-	-
IL-8	6	216.36	88.74–570.31	9	0.09	0.01–1.61	0	-	-	1	0.04	-	6	-	3	-	-	1
VEGF	2	1,931.72	878.45–2984.99	8	0.07	0.03–0.11	0	-	-	6	0.35	0.11–1.51	2	-	6	-	-	6
CXCL-1/2	4	118.77	17.25–266.48	2	0.98	0.61–1.35	0	-	-	0	-	-	2	2	-	-	-	-

Only values within the detection limits were included in the analysis. <sup>a</sup> Protein concentrations are presented in pg/mL. <sup>b</sup> Concentrations measured in the tape strip samples were normalized to the total amount of protein and presented as pg/ $\mu$ g protein. <sup>c</sup> Number of samples in which protein levels were within the detection limits. <sup>d</sup> Other levels were above the upper detection limit.

measurements within 1 patient since we did not want to miss any potential differences or correlations. Moreover, the marker set captured with the TAP was a preset general inflammatory panel, thus not specific for psoriasis. Additionally, a relatively low number of participants were included: 32 pediatric and 10 adult psoriasis patients. Given the daily practice setting, it was not feasible to study parameters for which a controlled setting is required (e.g., sensitivity or specificity). Lastly, the daily practice setting resulted in uncontrolled variables (such as treatment), possibly disturbing correlations between marker levels and psoriasis severity. However, it is important that correlations with disease severity are still present in a daily practice setting in order to be of value for clinical practice. Therefore, we underline the relevance of biomarker studies in a daily practice setting.

This study highlights important challenges for non-invasive biomarker measurements by TAP in pediatric psoriasis in a daily practice setting. Highly variable marker levels during the course of the disease were seen, and robust correlations between lesional marker levels measured by TAP and psoriasis severity could not be established. Moreover, detection issues related to desquamation were seen. Tape stripping may be considered instead of TAP for protein sampling of lesions with excessive desquamation, like in psoriasis. In the future, noninvasive biomarker measurements in daily practice could be of added value for managing psoriasis in the individual patient. However, in its current form, the use of biomarker measurements by TAP in pediatric psoriasis patients in daily clinical practice is still a bridge too far.

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## Statement of Ethics

This research was conducted in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was given by all participants aged  $\geq 16$  years. Participants aged 12–16 years gave written informed assent. Legal guardians of participants aged under 16 years gave written informed consent before enrollment. The study protocol was approved by the Ethical Committee of the region Arnhem-Nijmegen (NL60952.091.17 and NL73363.091.20).

## Conflict of Interest Statement

Mirjam J. Schaap has carried out clinical trials for Amgen, Celgene, Janssen, and Lilly and has acted as a paid speaker for Abbvie; fees were paid directly to the institution. Finola M. Bruins has carried out clinical trials for Abbvie, Amgen, Celgene, Janssen, Leo Pharma, Lilly, and Pfizer. Noa J.M. van den Brink has no conflicts of interest. Kadri Orro is employed by FibroTx. Hans M.M. Groenewoud has no conflicts of interest. Elke M.G.J. de Jong has received research grants for the independent research fund of the department of dermatology of the Radboud University Medical Center Nijmegen, The Netherlands, from AbbVie, Novartis, Janssen Pharmaceutica, Leo Pharma, and UCB for research in psoriasis. She has acted as consultant and/or paid speaker for and/or participated in research sponsored by companies that manufacture drugs used for the treatment of psoriasis or eczema including AbbVie, Almirall, Janssen Pharmaceutica, Novartis, Lilly, Celgene, Leo Pharma, Sanofi, UCB, and Galapagos. All funding is not personal but goes to the independent research fund of the Department of Dermatology of Radboud University Medical Center Nijmegen, The Netherlands. Ellen H. van den Bogaard has no conflicts of interest. Marieke M.B. Seyger received grants from/was involved in clinical trials for Abbvie, Amgen, Celgene, Eli Lilly, Janssen, Leo Pharma, and Pfizer. She served as a consultant for Abbvie, Eli Lilly, Janssen, Leo Pharma, Novartis, Pfizer, and UCB; fees were paid directly to the institution.

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## Author Contributions

Mirjam J. Schaap: contributed to data acquisition, data analysis, interpretation of data and wrote the manuscript. Finola M. Bruins: contributed to data acquisition, data analysis, interpretation of data, and manuscript revisions. Noa J.M. van den Brink: contributed to data acquisition, data analysis, and interpretation of data. Kadri Orro: contributed to research design, data analysis, interpretation of data, and manuscript revisions. Hans M.M. Groenewoud: contributed to data analysis, interpretation of data, and manuscript revisions. Elke M.G.J. de Jong: contributed to research design and manuscript revisions. Ellen H. van den Bogaard and Marieke M.B. Seyger: contributed to research design, interpretation of data, and manuscript revisions.

## Data Availability Statement

All analyses are included in this article or the supplemental materials. Further inquiries can be directed to the corresponding author.



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