Interleukin-8 Levels in the Stratum Corneum as a Biomarker for Monitoring Therapeutic Effect in Atopic Dermatitis Patients

Susumu Murata  Sakae Kaneko  Eishin Morita
Department of Dermatology, Shimane University Faculty of Medicine, Izumo, Japan

Keywords
Atopic dermatitis · Biomarker · Interleukin-8 · Stratum corneum · Tape-stripping method

Abstract
Introduction: The stratum corneum contains several growth factors and cytokines that are synthesized in keratinocytes. We previously reported that the amount of interleukin-8 in the stratum corneum (scIL-8) is related to the severity of local skin inflammation in atopic dermatitis (AD). However, it is unknown whether scIL-8 levels reflect pharmacologic responses to a therapeutic intervention in AD patients. Therefore, in this study, we aimed to investigate whether the improvement of dermatitis in AD is correlated with scIL-8 levels before and after topical corticosteroid treatment. Methods: Stratum corneum samples were collected from 22 AD patients using the noninvasive tape-stripping method before treatment, 2 weeks after topical treatment, and 4–6 weeks after treatment. Results: scIL-8 levels on the forearm reduced significantly from 790 ± 348 pg/mg before treatment to 163 ± 68 pg/mg 2 weeks after treatment and 100 ± 37 pg/mg 4–6 weeks after corticosteroid treatment. scIL-8 levels on the abdomen also reduced significantly from 902 ± 391 to 142 ± 38 pg/mg at the end of study. The reduction in scIL-8 levels was associated with the improvement in local skin severity in AD. We also found that scIL-8 levels, along with blood biomarker levels (serum thymus and activation-regulated chemokine, lactate dehydrogenase, and %eosinophil), decreased significantly after the treatment. Conclusion: The scIL-8 concentration decreases with improvements in skin symptoms in AD patients after topical corticosteroid treatment; thus, it may be a suitable biomarker for monitoring therapeutic effects in AD patients.

Introduction
Atopic dermatitis (AD) is a relapsing chronic inflammatory skin disorder that affects children and adults and is considered one of the most common chronic skin diseases, with an estimated global prevalence of 230 million [1, 2]. Several serum biomarkers have been used to evaluate the severity of AD. Of these, serum thymus and activation-regulated chemokine (TARC) is currently one of the most reliable biomarkers [3–5]. Serum lactate dehydrogenase (LDH) and eosinophil count are other biomarkers that correlate with AD severity [6]. Despite the
vital information that these serum biomarkers provide in the evaluation of AD, their measurement requires blood sampling; therefore, frequent measurements are not feasible. Recently, the tape-stripping technique was developed for noninvasive determination of the concentrations of cytokines and chemokines in the stratum corneum of cutaneous lesions [7]. Such measurements should reflect the inflammatory condition of the affected skin. Many cytokines and chemokines have been investigated for use as biomarkers of the severity of AD.

We previously reported that the amount of TARC in the stratum corneum (scTARC) is correlated with the severity of cutaneous lesions, especially the acute inflammatory signs, such as erythema, edema, papules, and oozing or crusts [8, 9]. scTARC is also correlated with the systemic severity of AD, as evaluated using the Severity Scoring of Atopic Dermatitis (SCORAD) index, serum TARC levels, serum total immunoglobulin E (IgE) levels, and blood eosinophil counts. However, scTARC is evaluated semi-quantitatively using an immunofluorescent technique as scTARC content is too low for quantification using an ELISA. As the immunofluorescent method is time and labor intensive, it is impractical for routine monitoring purposes.

Subsequently, we have used commercially available ELISAs to evaluate various cytokines and growth factors in the stratum corneum [10, 11]. We used the tape-stripping method for the noninvasive collection of stratum corneum samples and evaluated cytokines and growth factors that are considered to play a role in the inflammation of the skin. This included several interleukins (ILs); tumor necrosis factor-α; chemokine ligand 5 (RANTES); eotaxin; monocyte chemoattractant protein-1; macrophage inflammatory proteins-1α and 1β; granulocyte, macrophage, and granulocyte-macrophage colony-stimulating factor; nerve growth factor; vascular endothelial growth factor; and transforming growth factor (TGF)-α and TGF-β.

As a result, we discovered that IL-8, IL-18, vascular endothelial growth factor, and TGF-α were present in sufficient amounts to be measured using commercially available ELISAs and further evaluated their association with cutaneous symptoms [10, 11]. Of these cytokines, the amount of IL-8 in the stratum corneum (scIL-8) demonstrated the highest correlation coefficient with the cutaneous symptoms. Based on these observations, we speculated that scIL-8 level is a significant biomarker in evaluating cutaneous conditions as well as general disease severity in AD. However, whether scIL-8 concentration will reflect pharmacologic responses to AD symptom treatment remains unclear. Although several therapeutic options are available for the treatment of AD, the preferred first-line therapy is topical corticosteroid [12–14]. The aim of this study was to evaluate the changes in scIL-8 before and after topical corticosteroid treatment in patients with AD, to evaluate the correlation between change in scIL-8 level and improvements in skin symptoms, and to determine whether scIL-8 can be used as a biomarker to monitor disease activity in AD.

**Methods**

**Study Design and Patients**

We enrolled 22 patients (11 males and 11 females) from Shimane University Hospital who met the diagnostic criteria for AD established by the Japanese Dermatological Association [14]. Topical corticosteroid treatment was administered for 4–6 weeks (Fig. 1). Evaluation was performed at day 0 (first visit), 2 weeks later (second visit), and 4–6 weeks later (third visit), and blood examination was performed at the first and third visits. Patients undergoing systemic immunosuppressive therapy were excluded. This study was approved by the Ethics Committee of Shimane University Faculty of Medicine (Approval No. 1473) and was performed in accordance with the Declaration of Helsinki. The study design was fully explained to the patients, and written informed consent was obtained from them.

**Topical Treatment**

The AD patients were instructed to use daily topical corticosteroid ointments containing betamethasone butyrate propionate (Antebate®; Torii Pharmaceutical Co., Ltd, Tokyo, Japan). One fingertip unit of topical corticosteroid was suggested for use in an area of the skin twice the size of the palm of the patient’s hand. Depending on their symptoms, patients were allowed to use routine therapy including moisturizer ointment and antihistamines; however, no systemic treatment (oral corticosteroid or cyclosporine) was allowed during the test period.
Table 1. Background of patients

| Panelist | Age | Sex | Laboratory data | A | B | C | SCORAD |
|----------|-----|-----|-----------------|---|---|---|--------|
|          |     |     | TARC, pg/mL     | IgE, IU/mL | LDH, U/L | WBC <10^6/μL | eosinophil % | Affected lesions % | erythema | edema | exudation | scratch | lichenification | dryness | pruritus | sleepless |
| 1        | 31  | M   | 5,206           | 3,906      | 374      | 7.9          | 14.2         | 50            | 2   | 2   | 0   | 2   | 2   | 2   | 7   | 3   | 55 |
| 2        | 16  | F   | 5,920           | 1,341      | 516      | 10.2         | 27.9         | 30            | 1  | 1  | 0  | 2   | 2   | 1   | 9   | 8   | 48 |
| 3        | 34  | F   | 1,622           | 24,271     | 200      | 10.5         | 5.5          | 50            | 1  | 1  | 0  | 2   | 1   | 1   | 5   | 0   | 36 |
| 4        | 44  | M   | 2,979           | 15,955     | 415      | 6.6          | 8.0          | 42            | 2  | 1  | 1  | 1   | 3   | 3   | 4   | 2   | 53 |
| 5        | 25  | M   | 4,217           | 84.8       | 247      | 6.5          | 3.1          | 80            | 2  | 2  | 0  | 1   | 1   | 2   | 8   | 3   | 55 |
| 6        | 14  | F   | 1,324           | 803        | 215      | 8.8          | 8.8          | 40            | 1  | 1  | 0  | 1   | 1   | 2   | 8   | 8   | 42 |
| 7        | 38  | F   | 4,363           | 6,857      | 236      | 3.7          | 13.2         | 70            | 2  | 2  | 2  | 2   | 3   | 3   | 6   | 5   | 74 |
| 8        | 21  | M   | na              | na         | na       | na           | na           | 30            | 1  | 1  | 0  | 0   | 0   | 1   | 3   | 0   | 20 |
| 9        | 28  | F   | 37,910          | 24,200     | 286      | 6.0          | 3.5          | 32            | 3  | 2  | 0  | 3   | 2   | 3   | 7   | 5   | 64 |
| 10       | 37  | F   | 4,016           | 6,014      | 280      | 7.7          | 20.5         | 96            | 3  | 3  | 0  | 3   | 2   | 3   | 9   | 4   | 81 |
| 11       | 26  | F   | 3,091           | 524        | 228      | 7.0          | 5.7          | 15            | 1  | 0  | 0  | 0   | 0   | 0   | 1   | 8   | 4  |
| 12       | 28  | M   | 593             | 519        | 301      | 5.8          | 10.5         | 62            | 1  | 1  | 1  | 1   | 1   | 1   | 2   | 3   | 42 |
| 13       | 14  | M   | 6,123           | 728        | 286      | 5.7          | 36.3         | 40            | 2  | 2  | 0  | 2   | 2   | 0   | 2   | 7   | 5  |
| 14       | 24  | F   | 1,582           | 1,351      | 226      | 6.7          | 3.3          | 43            | 2  | 3  | 1  | 1   | 1   | 1   | 2   | 8   | 5  |
| 15       | 38  | F   | 1,120           | 41.9       | 213      | 5.2          | 5.2          | 29            | 2  | 1  | 2  | 1   | 1   | 1   | 2   | 4   | 0  |
| 16       | 17  | M   | 954             | 1,499      | 197      | 8.1          | 8.0          | 24            | 1  | 0  | 0  | 0   | 1   | 2   | 9   | 7   | 35 |
| 17       | 31  | M   | 5,377           | 10,587     | 390      | 9.5          | 11.8         | 90            | 2  | 2  | 1  | 2   | 2   | 2   | 2   | 7   | 6  |
| 18       | 19  | M   | 25,720          | 1,708      | 354      | 10.7         | 48.5         | 86            | 3  | 2  | 3  | 3   | 1   | 3   | 7   | 8   | 85 |
| 19       | 40  | M   | 35,330          | 720        | 327      | 6.7          | 7.4          | 68            | 3  | 2  | 2  | 2   | 2   | 0   | 2   | 7   | 5  |
| 20       | 20  | M   | 424             | 264        | 265      | 7.4          | 15.3         | 80            | 2  | 1  | 0  | 2   | 2   | 0   | 2   | 10  | 58 |
| 21       | 33  | F   | 657             | 5,873      | 208      | 7.3          | 5.6          | 20            | 2  | 2  | 0  | 1   | 2   | 2   | 3   | 0   | 39 |
| 22       | 49  | F   | 3,225           | 890        | 232      | 5.1          | 16.7         | 50            | 3  | 3  | 0  | 2   | 2   | 2   | 3.5 | 8   | 64 |
| Mean     | 28.5|     | 7,226           | 5,149      | 286      | 7.3          | 13.3         | 51.2          | 1.9 | 1.5| 0.6| 1.5 | 1.5 | 2.0 | 6.0 | 4.2 | 52.7 |
| SEM      | 2,367| 1,600| 18             | 0.4        | 2.5      | 5.0          | 0.2          | 0.2           | 0.2 | 0.2| 0.2| 0.2 | 0.2 | 0.5 | 0.7 | 3.7 |  

All patients’ serum laboratory data and SCORAD indexes were obtained before starting topical corticosteroid treatment. TARC, thymus and activation-regulated chemokine; IgE, immunoglobulin E; LDH, lactate dehydrogenase; SCORAD, Scoring Atopic Dermatitis; na, not available; SEM, standard error of the mean. The SCORAD index formula is: A/5 + 7B/2 + C. A is defined as the extent (0–100), B is defined as the intensity (0–18), and C is defined as the subjective symptoms (0–20). The maximum SCORAD score is 103.
Evaluation of Cutaneous Lesion Conditions

Three sites were chosen for the evaluations – the inside of the forearm, abdomen, and area with the most severe symptoms in each patient. Skin scores were assessed visually for each of the 3 skin sites to assess the severity of the disease using 7 SCORAD index parameters (erythema, edema, lichenification, oozing/exudation, excoriation, xerosis/dryness, and itch) [14]. According to increasing symptom severity, each parameter was scored from 0 to 3, for a total possible score of 21. Before tape stripping, transepidermal water loss (TEWL) and skin water content were measured at each skin site in an air-conditioned room using the Corneometer® CM825 and Tewameter® MPA5 (Courage & Khazaka electronic GmbH, Cologne, Germany), respectively.

Blood Examination
Blood was collected at the first and third visits to assess the white blood cell count, %eosinophil, serum levels of LDH, total IgE, and TARC.

Tape Stripping of the Stratum Corneum
Tape stripping was performed on the cutaneous sites using plastic tape (24 mm × 5 cm, Cellotape®; Nichiban, Tokyo, Japan) [10, 11] after the sites were cleaned with ethanol. Plastic tape was

---

![Graphs showing correlation between scIL-8 concentration and skin score, TEWL, and skin water content for forearm, abdomen, and area with the most severe symptoms.](image-url)
applied to the skin, pressed for approximately 10 s, and removed gently; the same procedure was repeated 5 times. The pieces of tape were stored at −20°C until further analysis.

**Measurement of scIL-8**

scIL-8 was evaluated using the method described previously [10, 11]. The tape-stripping samples were briefly immersed in 5 mL of hexane. After centrifugation (3,000 rpm, 15 min at 4°C), the supernatant, containing tape glue and miscellaneous chemicals, was removed. The remaining samples were again subjected to centrifugation (15,000 rpm, 15 min at 4°C) followed by the addition of 1 mL of hexane. The precipitants, containing the corneal layers, were collected. Proteins were extracted in 1 mL of extraction buffer (0.1 M Tris-HCl, pH 8.0, and 0.5% Triton X-100) under ultrasound sonification (Branson Sonifier® 450; Emerson Japan, Ltd., Atsugi, Japan) for 3 min. The supernatants were purified using 4-mm filters (Millex®; Millipore, Tokyo, Japan) and subjected to centrifugation (15,000 rpm, 15 min at 4°C). scIL-8 in the purified supernatants was analyzed using ELISA kits (Human IL-8/CXCL8 Quantikine® ELISA; R&D Systems, Minneapolis, MN, USA). The total protein contents were measured using the DC protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). scIL-8 concentration was expressed as pg per mg of protein content of the stratum corneum.

**Fig. 3.** Change in skin scores before, during, and after topical treatment. Skin scores were evaluated at the first, second, and third visits for the forearm, abdomen, and area with the most severe symptoms (others*). The average data are indicated by the bar graphs at the top, and individual data are indicated by the line graphs below. Data are expressed as the mean ± SEM. **p < 0.01, ***p < 0.001. SEM, standard error of the mean.
**Statistical Analysis**

Student’s *t* test and Mann-Whitney’s U test were used to compare scIL-8 levels between the 2 groups, and Spearman’s rank correlation test was used to calculate the correlations. Results are expressed as the mean ± standard error of the mean, unless otherwise indicated. The results were considered to be significantly different or correlated when the *p* value <0.05.

**Results**

**Patient Demographics and Clinical Characteristics**

The mean ± standard deviation age of the overall cohort was 28.5 ± 9.9 years. Of the 22 patients, 13 had severe symptoms (SCORAD >50), 7 had moderate symptoms (SCORAD 25–50), and 2 had mild symptoms (SCORAD <25). The mean ± standard deviation SCORAD score was 52.6 ± 17.0 (Table 1).
Correlation between scIL-8 and Skin Scores, TEWL, and Skin Water Content before Topical Treatment

The average scIL-8 concentration in the patients before the treatment was 790 ± 348 pg/mg on the forearm, 902 ± 391 pg/mg on the abdomen, and 1,905 ± 500 pg/mg over the lesions with the most severe symptoms. The correlation between scIL-8 and skin scores at the 3 sites is illustrated in Fig. 2a–c. Significant correlations were observed between scIL-8 and skin score on the forearm ($r_s = 0.50$, $p < 0.001$), abdomen ($r_s = 0.37$, $p < 0.01$), and area with the most severe symptoms ($r_s = 0.53$, $p < 0.001$). The correlation between scIL-8 and TEWL in the same areas before topical treatment is illustrated in Fig. 2d–f. A significant correlation was observed between scIL-8 and TEWL in the forearm ($r_s = 0.45$, $p < 0.05$), abdomen ($r_s = 0.69$, $p < 0.01$), and area with the most severe symptoms ($r_s = 0.42$, $p < 0.05$). However, no statistically significant correlation was found between scIL-8 and skin water content in the same areas (Fig. 2g–i).

Fig. 5. Changes in skin water content before, during, and after topical treatment. Skin water content was evaluated at the first, second, and third visits for the forearm, abdomen, and area with the most severe symptoms (others*). The average data are indicated by the bar graphs at the top, and individual data are indicated by the line graphs below. Data are expressed as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SEM, standard error of the mean.

Correlation between scIL-8 and Skin Scores, TEWL, and Skin Water Content before Topical Treatment

The average scIL-8 concentration in the patients before the treatment was 790 ± 348 pg/mg on the forearm, 902 ± 391 pg/mg on the abdomen, and 1,905 ± 500 pg/mg over the lesions with the most severe symptoms. The correlation between scIL-8 and skin scores at the 3 sites is illustrated in Fig. 2a–c. Significant correlations were observed between scIL-8 and skin score on the forearm ($r_s = 0.50$, $p < 0.001$), abdomen ($r_s = 0.37$, $p < 0.01$), and area with the most severe symptoms ($r_s = 0.53$, $p < 0.001$). The correlation between scIL-8 and TEWL in the same areas before topical treatment is illustrated in Fig. 2d–f. A significant correlation was observed between scIL-8 and TEWL in the forearm ($r_s = 0.45$, $p < 0.05$), abdomen ($r_s = 0.69$, $p < 0.01$), and area with the most severe symptoms ($r_s = 0.42$, $p < 0.05$). However, no statistically significant correlation was found between scIL-8 and skin water content in the same areas (Fig. 2g–i).

Fig. 5. Changes in skin water content before, during, and after topical treatment. Skin water content was evaluated at the first, second, and third visits for the forearm, abdomen, and area with the most severe symptoms (others*). The average data are indicated by the bar graphs at the top, and individual data are indicated by the line graphs below. Data are expressed as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SEM, standard error of the mean.
Skin Score, TEWL, and Skin Water Content before, during, and after Topical Treatment

All 22 patients completed this study. The average skin score, TEWL, and skin water content before, during, and after topical treatment are shown in Figures 3–5. Skin scores decreased significantly at the second and third visits compared to those at the first visit at all 3 sites (Fig. 3). Additionally, the average TEWL values decreased significantly at the second and third visits compared to those at the first visit at all 3 sites (Fig. 4). Skin water content increased significantly at the third visit compared to those at the first visit at all 3 sites (Fig. 5). The actual average skin score, TEWS, and skin water content throughout the test period are summarized in Table 2.

Changes in Laboratory Parameters before and after Topical Treatment

The mean serum levels of TARC, total IgE, LDH, and %eosinophil before and after the topical treatment are summarized in Figure 6. The serum levels of TARC and LDH decreased significantly at the third visit, whereas that of serum total IgE did not change significantly. The blood %eosinophil decreased significantly at the third visit.

Correlation between scIL-8 Reduction and Skin Score Improvement with Topical Treatment

The correlation between the reduction in scIL-8 levels (ΔscIL-8) and the degree of improvements in the skin score (Δskin score) following the topical treatment is shown in Figure 8. ΔscIL-8 (difference between the values at first and third visits) was significantly correlated with the Δskin score (difference between the values at first and third visits) in the forearm (rₛ = 0.50, p < 0.01), abdomen (rₛ = 0.82, p < 0.001), and area with the most severe symptoms (rₛ = 0.55, p < 0.01). Similar significant correlations were observed between the ΔscIL-8 (difference between the values at first and second visits) and the Δskin score (difference between the values at first and second visits) for all 3 sites, and between the ΔscIL-8 (difference in the values at second and third visits) and Δskin score (difference in the values at second and third visits) for the abdomen and area with the most severe symptoms (Fig. 8).

Correlation between scIL-8 Reduction and Improvements in TEWL and Skin Water Content following Topical Treatment

The correlation between ΔscIL-8 and the degrees of improvement in TEWL (ΔTEWL) and skin water content (Δskin water content) following topical treatment is illustrated in Figure 9. When the ΔscIL-8 and ΔTEWL were analyzed between the first and third visits, there were no significant correlations in the forearm (rₛ = 0.16), abdomen (rₛ = 0.33), or area with the most severe symptoms (rₛ = 0.21). However, when ΔscIL-8 and Δskin water content were analyzed between the first and third visits, a significant correlation was observed in the abdomen (rₛ = 0.41, p < 0.05).

Correlation between Reduction in scIL-8 and Improvements in the General Severity Parameters following Topical Treatment

The correlation between ΔscIL-8 and the improvements in serum levels of TARC (ΔTARC), %eosinophil (Δ%eosinophil), and LDH (ΔLDH) following topical treat-
ment is presented in Figure 10. Significant correlations were noted between ΔscIL-8 and ΔTARC in the forearm ($r_s = 0.65$, $p < 0.01$) and abdomen ($r_s = 0.53$, $p < 0.01$), between ΔscIL-8 and Δ%eosinophil in the abdomen ($r_s = 0.50$, $p < 0.05$), and between ΔscIL-8 and ΔLDH in the forearm ($r_s = 0.39$, $p < 0.05$) and abdomen ($r_s = 0.54$, $p < 0.01$). No significant correlations were noted between ΔscIL-8 and improvement in serum IgE levels (data not shown).

**Discussion/Conclusion**

This study demonstrated that scIL-8, measured using the tape-stripping method, reflected the response to topical corticosteroid therapy in AD patients; further, the degree of change in scIL-8 concentration was correlated with visual improvements in symptoms.

Before the topical corticosteroid treatment, the scIL-8 concentration at lesion sites correlated with the visual skin score, which is consistent with the previous observations by McAleer et al. [15] and Hulshof et al. [16], as well as with our previous results [11]. McAleer et al. [15] reported that 19 cytokines, including IL-8, demonstrated significant differences between healthy subjects and infants with AD; additionally, they showed that the levels of IL-8 and IL-18 were the highest among cytokines measured in the stratum corneum. Hulshof et al. [16] demonstrated that IL-8, CCL2, and TARC measured using the tape-stripping method in children with AD showed an association in the objective SCORAD score. These cumu-
Relative findings suggest that assessment of scIL-8 is a useful tool in evaluating the severity of skin inflammation in AD patients; however, data on the change in scIL-8 level with pharmaceutical intervention are lacking. Topical corticosteroid treatment is the preferred first-line therapy for AD, as recommended in the guidelines by the Japanese, American, and European Academies of Dermatology [12–14]. Koppes et al. [17] investigated the effects of 6 weeks of ceramide- and magnesium-containing emollient therapy on 38 inflammatory mediators in the stratum corneum in mild and moderate AD patients. They reported that decreases in TARC and IL-8 were correlated with the decrease of disease severity in the subgroup of moderate AD individuals. In their study, patients with severe AD were excluded, and patients were not allowed to apply topical corticosteroids. In the present study, we demonstrated that changes in scIL-8 levels reflect pharmacologic responses to topical corticosteroids for improvement of clinical AD symptoms. To the best of our knowledge, this study is the first to demonstrate the usefulness of scIL-8 determination in evaluating improvements of skin lesions in patients with AD through daily topical corticosteroid treatment. Following topical corticosteroid treatment, skin score improved significantly, as
indicated in Figures 3, and the sIL-8 level decreased significantly, as indicated in Figure 7. In addition, the degree of skin symptom improvement (Δskin score) was correlated with ΔsIL-8 (Fig. 8). It is noteworthy that the higher correlation coefficients were observed between ΔsIL-8 and the Δskin score upon subgroup analysis of patients with severe AD (SCORAD >50, n = 14) between the first and third visits. The $r_s$ values were as follows: forearm, 0.63 ($p < 0.01$); abdomen, 0.80 ($p < 0.01$); and area with the most severe symptoms, 0.73 ($p < 0.01$) (data not shown). This stronger correlation in the subgroup with severe AD is consistent with the study done by Koppes et al. [17] with topical emollient treatment. In our previous study, we described that sIL-8 correlates highly with acute phase symptoms, such as erythema, edema/papules, and excoriation; however, it is weaker with chronic phase symptoms, such as lichenification and oozing/crust [11]. This could be one of the potential reasons why correlation between sIL-8 level and visual skin score is low in mild AD where chronic phase symptoms are predomin-
interleukin-8 level and visual skin score is high in severe AD patients where acute phase symptoms are predominant.

As we have previously reported, scIL-8 levels are extremely low in persons without AD – almost under the detection limit of the commercially available ELISA kit; in comparison, such levels are increased up to 100 times and more in patients with AD [10, 11]. This is in agreement with the present results, in which all patients with AD demonstrated detectable levels of scIL-8 on the forearm, abdomen, and skin affected worst with symptoms. Paralleling improvement in skin symptoms, scIL-8 levels drastically decreased after 2 weeks of topical treatment and remained low until at least 4–6 weeks of treatment (Fig. 5). It should be noted that scIL-8 levels were still detectable after 4–6 weeks of topical treatment in most patients. Only 2/22, 2/22, and 2/22 patients did not demonstrate detectable levels of scIL-8, respectively, on the forearm, abdomen, and the lesion sites with the most severe symptoms. For these sites, there were 7/22, 6/22, and 5/22 patients, respectively, with a skin score of 0. These results suggest that scIL-8 has high sensitivity to reflect improvements in local inflammation in patients with AD, more so than visual skin scoring.

We discovered that scIL-8 was weakly correlated with TEWL, not with skin water content (Fig. 2), although our previous findings demonstrated that scIL-8 was associated with both TEWL and skin water content. This might be due to the number of patients investigated: 22 in this study compared to 55 in the previous study [11]. ΔscIL-8 did not show a correlation with ΔTEWL or Δskin water content in this study, thus suggesting that scIL-8 may not be a sensitive biomarker in evaluating the improvements in barrier damage due to AD.

Additionally, scIL-8 might reflect systemic disease severity of AD, especially when it is evaluated on the forearm or abdomen, since ΔscIL-8 was correlated with serum ΔTARC and ΔLDH levels, which are established biomarkers of severity in AD (Fig. 10). During topical treatment, no significant change was observed in total serum IgE levels although serum levels of TARC, LDH, and %eosinophil declined significantly (Fig. 6). This is consis-
tent with the findings of previous studies, in which it was reported that total serum IgE levels correlated with the severity of AD but did not decrease proportionally with improvements in AD [18–20]. Although scIL-8 level correlated significantly above serum biomarkers, these correlation coefficients were relatively low. It may be a reasonable assumption that scIL-8 serves as biomarker for local skin severity of AD more than systemic inflammation, whereas serum blood markers reflect more systemic inflammation in AD patients.

The tape-stripping technique has been established as a noninvasive and relatively quick and simple method for estimating cytokine concentrations in the stratum corneum [21–25]. In this study, we successfully obtained the stratum corneum from the lesions before, during, and after topical treatment.

IL-8 is a pro-inflammatory chemokine and a potent chemoattractant for neutrophils, playing a role in the activation of the innate immunity. IL-8 was identified originally as a neutrophil-activating peptide or human monocyte-derived neutrophil chemotactic factor from super-
Interleukin-8 in Stratum Corneum as a Biomarker of Atopic Dermatitis

Acknowledgements

We thank all the participating medical practices for the recruitment of patients and for their support of this clinical study.

Statement of Ethics

This study was approved by the Ethics Committee of Shimane University Faculty of Medicine (Approval No. 1473) and was performed in accordance with the Declaration of Helsinki. The study design was fully explained to the patients, and written informed consent was obtained from them.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The authors did not receive any funding.

Author Contributions

All authors contributed to editing and reviewing of the draft manuscript and provided approval of the final version of the manuscript.

References

1 Tsai TF, Rajagopalan M, Chu CY, Encarnacion L, Gerber RA, Santos-Estralla P, et al. Burden of atopic dermatitis in Asia. J Dermatol. 2019 Oct; 46(10):825–34.
2 Silvestre Salvador JF, Romero-Pérez D, Encabo-Durán B. Atopic dermatitis in adults: a diagnostic challenge. J Investig Allergol Clin Immunol. 2017;27(2):78–88.
3 Tamaki K, Kakinuma T, Saeli H, Horikawa T, Katoaka Y, Fujisawa T, et al. Serum levels of CCL17/TARC in various skin diseases. J Dermatol. 2006 Apr;33(4):300–2.
4 Jahnz-Rozyk K, Targowski T, Paluchowska E, Andrzejewski M, Jankowska-Sadowska M, et al. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. Curr Opin Allergy Clin Immunol. 2015 Oct;15(5):453–60.
5 Aoki T, Yoshida H, Furue M, Tagami H, Kaneko F, Ohtsuka F, et al. English version of the Japan Dermatological Association. J Dermatol. 2011 Jul;38(11):116–32.
6 Morishima Y, Kawashima H, Takekuma K, Hoshika A. Changes in serum lactate dehydrogenase activity in children with atopic dermatitis. Pediatr Int. 2010 Apr;52(4):477–81.
7 Perkins MA, Osterhues MA, Farage MA, Robinson MK. A noninvasive method to assess skin irritation and compromised skin conditions using simple tape adsorption of molecular markers of inflammation. Skin Res Technol. 2001 Nov;7(4):227–37.
8 Morita E, Hiragun T, Mihara S, Kaneko S, Matsuo H, Zhang Y, et al. Determination of thymus and activation-regulated chemokine (TARC)-content in scales of atopic dermatitis. J Dermatol Sci. 2004 May;34(3):237–40.
9 Morita E, Takahashi H, Niihara H, Dekio I, Sumikawa Y, Murakami Y, et al. Stratum corneum TARC level is a new indicator of lesion-related skin inflammation in atopic dermatitis. Allergy. 2010 Sep;65(9):1166–72.
10 Amarbayasgalan T, Takahashi H, Dekio I, Morita E. Content of vascular endothelial growth factor in stratum corneum well correlates to local severity of acute inflammation in patients with atopic dermatitis. Int Arch Allergy Immunol. 2012;157(3):251–8.
11 Amarbayasgalan T, Takahashi H, Dekio I, Morita E. Interleukin-8 content in the stratum corneum as an indicator of the severity of inflammation in the lesions of atopic dermatitis. Int Arch Allergy Immunol. 2013;160(1):63–74.
12 Wollenberg A, Barbarot S, Bieber T, Christen-Zaech S, Deleuran M, Fink-Wagner A, et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I. J Eur Acad Dermatol Venereol. 2018 May;32(5):657–82.
13 Ichinoshita K, Tsuchida H, Ohara S, Tomioka T, Kato H, Kato A, et al. Guidelines for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. J Am Acad Dermatol. 2014 Jul;71(1):116–32.
14 Aoki T, Yoshida H, Furue M, Tagami H, Kaneko F, Ohitsuuka F, et al. English version of the concluding report published in 2001 by the Advisory Committee on Atopic Dermatitis Severity Classification Criteria of the Japanese Dermatological Association. J Dermatol. 2011 Jul;38(7):632–44.
15 McAleer MA, Jakasa I,Hurault G, Sarvari P, McLean WHI, Tanaka RJ, et al. Systemic and stratum corneum biomarkers of severity in infant atopic dermatitis include markers of innate and T helper cell-related immunity and angiogenesis. Br J Dermatol. 2019 Mar; 180(3):586–96.

16 Hulshof L, Hack DP, Hasnou QCJ, Donjtse B, Jakasa I, Riethmüller C, et al. A minimally invasive tool to study immune response and skin barrier in children with atopic dermatitis. Br J Dermatol. 2019 Mar;180(3):621–30.

17 Koppes SA, Brans R, Ljubojevic Hadzavdic S, Frings-Dresen MH, Rustemeyer T, Kezic S. Stratum corneum tape stripping: monitoring abnormalities in the skin of children with ear-nescent atopic dermatitis. JAMA Dermatol. 2019 Oct 9; 155(12): 1358–70.

18 Stevens SR, Hanifin JM, Hamilton T, Tofte SJ, Cooper KD. Long-term effectiveness and safety of recombinant human interferon gamma-4 therapy for atopic dermatitis. Arch Dermatol. 1998 Jul; 134(7):799–804.

19 Kou K, Aihara M, Matsunaga T, Chen H, Thijs JL, van Seggelen W, Bruijnzeel-Koomen C, de Bruin-Weller M, Hijnen D. New developments in biomarkers for atopic dermatitis. Int Arch Allergy Immunol. 2016;170(3):187–93.

20 Sticherling M, Bornscheuer E, Schröder JM, Christophers E. Immunohistochemical studies on NAP-1/IL-8 in contact eczema and atopic dermatitis. Arch Dermatol Res. 992; 284(2):82–5.

21 Akdis CA, Akdis M. Immunological differences between intrinsic and extrinsic types of atopic dermatitis. Clin Exp Allergy. 2003 Dec; 33(12):1618–21.

22 Guttman-Yassky E, Diaz A, Pavel AB, Fernandez M, Lefferdink R, Erickson T, et al. Use of tape strips to detect immune and barrier abnormalities in the skin of children with early-onset atopic dermatitis. JAMA Dermatol. 2019 Oct 9;155(12):1358–70.

23 Kezic S, Kammeyer A, Calkoen F, Fluhr JW, Bos JD. Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. Br J Dermatol. 2009 Nov; 161(5):1098–104.

24 Breternitz M, Flach M, Präßler J, Elsner P, Fluhr JW. Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping. A randomized, controlled study. Br J Dermatol. 2007 Feb;156(2):231–40.

25 de Jongh CM, Verberk MM, Spiekstra SW, Gibbs S, Kezic S. Cytokines at different stratum corneum levels in normal and sodium lauryl sulphate-irritated skin. Skin Res Technol. 2007 Nov;13(4):390–8.

26 Schröder JM, Mrowietz U, Morita E, Christophers E. Localization of neutrophil-activating peptide-1/interleukin-8 in psoriatic scales. J Invest Dermatol. 1991 Jan;96(1):26–30.

27 Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, et al. Molecular cloning of a human monocyte-derived neutrophil-activating peptide that lacks interleukin 1 activity. J Immunol. 1987 Nov 15;139(10):3474–83.

28 Kuzuhara S, Morita S, et al. Association of se-tin-8. Am J Respir Cell Mol Biol. 1990 Jun;5(4):430–2.

29 Peveri P, Walz A, Dewald B, Baggiolini M. A novel neutrophil-activating factor produced by human mononuclear phagocytes. J Exp Med. 1988 May 1;167(5):1547–59.

30 Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-acti-vating protein (NAP-1) is also chemotactic for T lymphocytes. Science. 1989 Mar 17; 243(4897):1464–6.

31 Leonard EJ, Yoshimura T. Neutrophil attractant/activation protein-1 (NAP-1 [interleu-kin-8]). Ann Intern Med. 1990 Jul 1;113(1):53–8.

32 Schröder JM, Bornscheuer E, Schröder JM, Christophers E. Neutrophil-activating factor-1/ interleukin-8-immuno-reactivity in normal and psoriatic skin. J Invest Dermatol. 1991 Jan;96(1):26–30.

33 Schröder JM, Gregory H, Young J, Christophers E. Neutrophil-activating proteins in psoriasis. J Invest Dermatol. 1992 Feb;98(2):241–7.

34 Schröder JM, Gregory H, Young J, Christophers E. Localization of neutrophil-activating peptide-1/interleukin-8-immuno-reactivity in normal and psoriatic skin. J Invest Dermatol. 1991 Jan;96(1):26–30.

35 Nygaard U, Vestergaard C, Deleuran M. Emerging treatment options in atopic derma-titis: systemic therapies. Dermatologia. 2017; 233(Suppl 3):44–57.

36 Broeders JA, Ahmed Ali U, Fischer G. Systematic review and meta-analysis of randomized clinical trials (RCTs) comparing topical calcineurin inhibitors with topical corticoste-roids for atopic dermatitis: a 15-year experi-ence. J Am Acad Dermatol. 2016 Aug;75(2): 410–9.e3.