Research Article

Interleukin-8 levels in the stratum corneum as a biomarker for monitoring therapeutic effect in atopic dermatitis patients

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Short Title: Interleukin-8 in stratum corneum as a biomarker of atopic dermatitis

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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 pg/mg 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 [TARC], serum lactate dehydrogenase [LDH], 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 enzyme-linked immunosorbent assay (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 (VEGF); and transforming growth factor (TGF)-α and TGF-β. As a result, we discovered that IL-8, IL-18, VEGF, 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 and to evaluate the correlation between change in scIL-8 level and improvements in skin symptoms, 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 Ethical 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, 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.

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 three skin sites to assess the severity of the disease using seven 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 applied to the skin, pressed for approximately 10 seconds, and removed gently; the same procedure was repeated five 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 (3000 rpm, 15 min at 4 °C), the supernatant, containing tape glue and miscellaneous chemicals, was removed. The remaining samples were again subjected to centrifugation (15000 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 (15000 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.

Statistical analysis

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

Results

Patient demographics and clinical characteristics

The mean ± standard deviation (SD) 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 ± SD 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 1905 ± 500 pg/mg over the lesions with the most severe symptoms. The correlation between scIL-8 and skin scores at the three sites is

illustrated in Fig. 2a–c. Significant correlations were observed between scIL-8 and skin score on the forearm (rs = 0.50, P<0.001), abdomen (rs = 0.37, P <0.01), and area with the most severe symptoms (rs = 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 (rs=0.45, P<0.05), abdomen (rs=0.69, P<0.01), and area with the most severe symptoms (rs=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).

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 Fig. 3-5. Skin scores decreased significantly at the second and third visits compared to those at the first visit at all three 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 three sites (Fig. 4). Skin water content increased significantly at the third visit compared to those at the first visit at all three 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 Fig. 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.

Changes in scIL-8 before and after topical treatment

The average levels of scIL-8 before, during, and after topical treatment are presented in Fig. 7 and Table 2. scIL-8 levels on the forearm, abdomen, and on the skin lesion with the most severe symptoms decreased significantly from the first visit to the second and third visits. The highest reduction in scIL-8 levels was between the first and third visits on the skin lesion with the most severe symptom.

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 Fig. 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 three 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 the ΔscIL-8 and the degrees of improvement in TEWL (ΔTEWL) and skin water content (Δskin water content) following topical treatment is illustrated in Fig. 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 treatment is presented in Fig. 10. Significant correlations were noted between ΔscIL-8 and ΔTARC in the forearm (rs = 0.65, $P<0.01$) and abdomen (rs = 0.53, $P<0.01$), between ΔscIL-8 and Δ%eosinophil in the abdomen (rs = 0.50, $P<0.05$), and between ΔscIL-8 and ΔLDH in the forearm (rs = 0.39, $P<0.05$) and abdomen (rs = 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. and Hulshof et al., 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 cumulative 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 is 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 Fig. 3, and the scIL-8 level decreased significantly, as indicated in Fig. 7. In addition, the degree of skin symptom improvement (Δskin score) was correlated with ΔscIL-8 (Fig. 8). It is noteworthy that the higher correlation coefficients were observed between ΔscIL-8 and the Δskin score upon subgroup analysis of patients with severe AD (SCORAD>50, n=14) between the first and third visits. The rs 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 severer AD is consistent with the study done by

Koppes et al. [17] with topical emollient treatment. In our previous study, we described that scIL-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 scIL-8 level and visual skin score is low in mild AD where chronic phase symptoms are predominant and correlation between scIL-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 consistent 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, whereus 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 supernatants obtained from activated monocytes [26-29]. This monokine was demonstrated to have chemotactic activity for T lymphocytes and was later renamed as IL-8 [30,31]. The production of large amounts of IL-8 in psoriasis has also been demonstrated repeatedly by Schröder et al. [32-35]. Thus, scIL-8 is not specific to AD and could be used to monitor other

diseases related to skin inflammation. Therefore, the measurement of scIL-8 using the tape-stripping method may also be applied in evaluating improvements in the severity of lesions in psoriasis or other inflammation-related skin diseases.

The limitations of this study are its relatively small sample size (n=22) and the absence of potent pharmaceutical treatments other than topical corticosteroid treatment. While topical corticosteroid therapy has been the standard therapy for AD, other treatments including topical calcineurin inhibitors, systemic oral cyclosporine, and dupilumab injection therapy can be used to treat AD. The present study did not demonstrate a change in scIL-8 levels secondary to these therapies or other emerging treatments, including those that mitigate the JAK-STAT pathway and PDE4 enzyme inhibition [36, 37].

In conclusion, the degree of scIL-8 change, estimated using the noninvasive tape-stripping method, reflects improvement in skin symptoms following first-line AD treatment with topical corticosteroids. Thus, it may be a valuable biomarker to monitor therapeutic effect in AD patients.

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Statement of Ethics

This study was approved by the Ethical Committee of Shimane University Faculty of Medicine (Approval No. 1473).

Conflict of Interest

The authors have no conflicts of interest to declare.

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Author Contributions

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

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Figure Legends

Fig. 1.

Study design. *Evaluation included skin score, transepidermal water loss, and skin water content. **Blood examination includes white blood cell count, %eosinophil, serum lactate dehydrogenase, serum thymus and activation-regulated chemokine, and serum total immunoglobulin E.

Fig. 2.

Pre-treatment correlations in atopic dermatitis patients between stratum corneum interleukin-8 (scIL-8) concentration and skin score, transepidermal water loss (TEWL), and skin water content of the forearm, abdomen, and area with the most severe symptoms (others*). There were significant correlations between scIL-8 and skin score and between scIL-8 and TEWL, but not between scIL-8 and skin water content.

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 is indicated by the bar graphs at the top, and individual data is indicated by the line graphs below. Data are expressed as the mean ± standard error of the mean. **P<0.01, ***P<0.001.

Fig. 4.

Changes in transepidermal water loss (TEWL) before, during, and after topical treatment. TEWL was evaluated at the first, second, and third visits for the forearm, abdomen, and area with the

most severe symptoms (others*). The average data is indicated by the bar graphs at the top, and individual data is indicated by the line graphs below. Data are expressed as the mean ± standard error of the mean. *P<0.05, ***P<0.001.

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 is indicated by the bar graphs at the top, and individual data is indicated by the line graphs below. Data are expressed as the mean ± standard error of the mean. *P<0.05, **P<0.01, ***P<0.001.

Fig. 6.

Laboratory data before and after treatment. Levels of serum thymus and activation-regulated chemokine (TARC), serum total immunoglobulin E (IgE), serum lactate dehydrogenase (LDH), and blood %eosinophil were evaluated at the first and third visits. *P<0.05, **P<0.01.

Fig. 7.

Changes in stratum corneum interleukin-8 (scIL-8) level before, during, and after topical treatment. scIL-8 was evaluated at the first, second, and third visits on the forearm, abdomen, and area with the most severe symptoms (others*). The average data is indicated in the bar graphs at the top, and individual data is indicated in the line graphs below. Significant reductions in scIL-8 levels were observed following topical corticosteroid treatment at all three skin sites. Data are expressed as the mean ± standard error of the mean. *P<0.05, **P<0.01.

Fig. 8.

Correlation between delta stratum corneum interleukin-8 (scIL-8) and delta skin score at the forearm, abdomen, and area with the most severe symptoms (others*). The values were calculated at the first to third visits, first to second visits, and second to third visits. NS, not significant.

Fig. 9.

Correlation between delta stratum corneum interleukin-8 (scIL-8) and delta transepidermal water loss (TEWL) and delta skin water content, respectively, at the forearm, abdomen, and the area with the most severe symptoms (others*) for the first to third visits. NS, not significant.

Fig. 10.

Comparison between delta stratum corneum interleukin-8 (scIL-8) and delta serum levels of thymus and activation-regulated chemokine (TARC), %eosinophil, and lactate dehydrogenase (LDH) at the forearm, abdomen, and area with the most severe symptoms (others*) for the first to third visits. Significant correlations were observed between delta scIL-8 and delta TARC, delta %eosinophil, and delta LDH. NS, not significant.

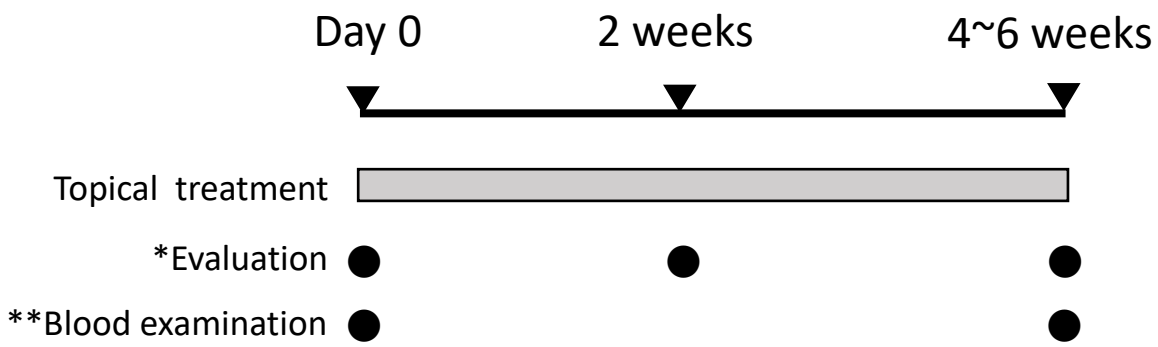
Day 0
2 weeks
4~6 weeks
Topical treatment
*Evaluation
**Blood examination

Fig. 1

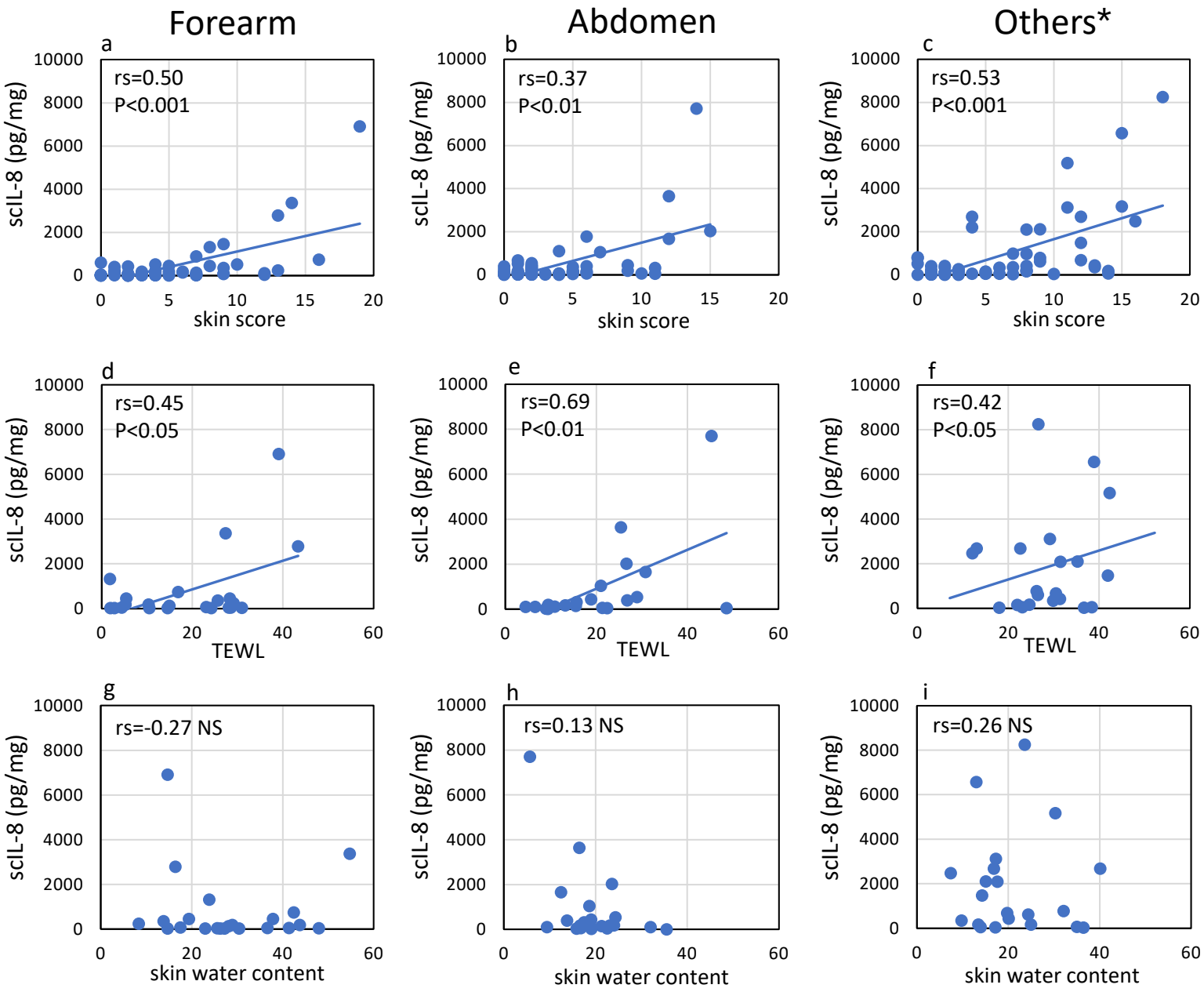
Forearm
Abdomen
Others*
a
b
c
d
e
f
g
h
i
scIL-8 (pg/mg)
skin score
TEWL
skin water content
rs=0.50
P<0.001
rs=0.37
P<0.01
rs=0.53
P<0.001
rs=0.45
P<0.05
rs=0.69
P<0.01
rs=0.42
P<0.05
rs=-0.27 NS
rs=0.13 NS
rs=0.26 NS

Fig. 2

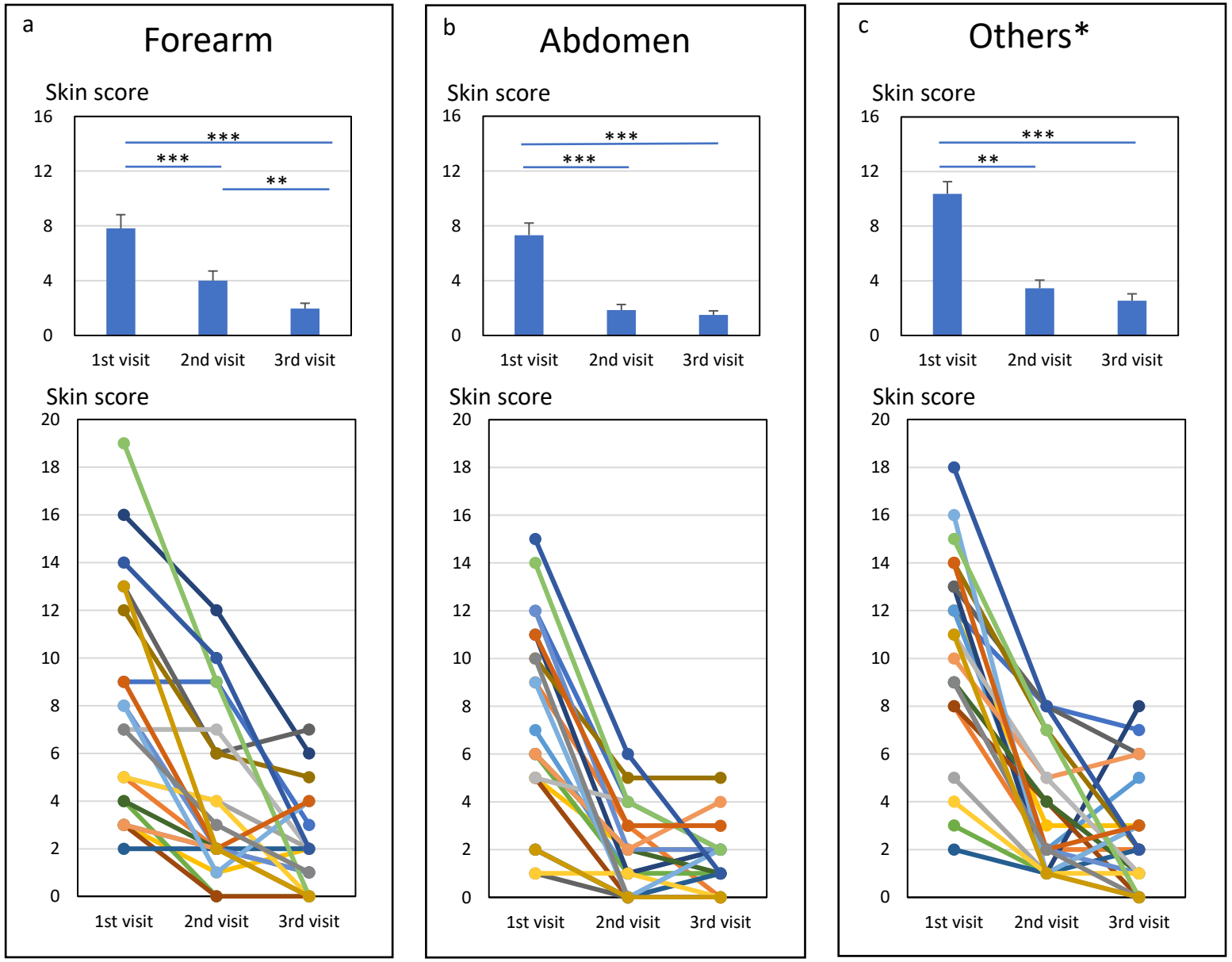
a
Forearm
Skin score
16
12
8
4
0
***
***
**
1st visit
2nd visit
3rd visit
Skin score
20
18
16
14
12
10
8
6
4
2
0
1st visit
2nd visit
3rd visit
b
Abdomen
Skin score
16
12
8
4
0
***
***
1st visit
2nd visit
3rd visit
Skin score
20
18
16
14
12
10
8
6
4
2
0
1st visit
2nd visit
3rd visit
c
Others*
Skin score
16
12
8
4
0
***
**
1st visit
2nd visit
3rd visit
Skin score
20
18
16
14
12
10
8
6
4
2
0
1st visit
2nd visit
3rd visit

Fig. 3

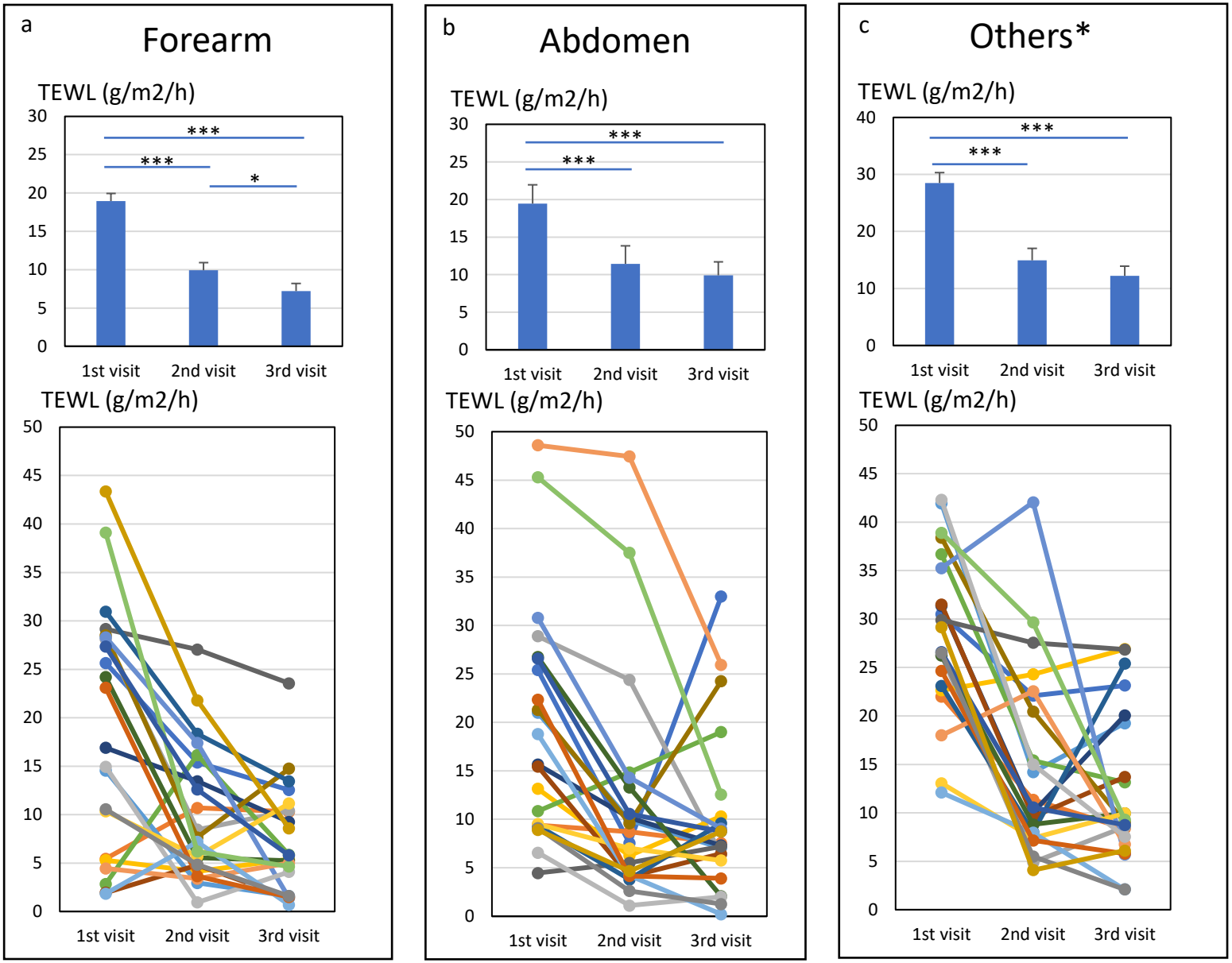
a
Forearm
TEWL (g/m2/h)
30
25
20
15
10
5
0
***
***
*
1st visit
2nd visit
3rd visit
TEWL (g/m2/h)
50
45
40
35
30
25
20
15
10
5
0
1st visit
2nd visit
3rd visit
b
Abdomen
TEWL (g/m2/h)
30
25
20
15
10
5
0
***
***
1st visit
2nd visit
3rd visit
TEWL (g/m2/h)
50
45
40
35
30
25
20
15
10
5
0
1st visit
2nd visit
3rd visit
c
Others*
TEWL (g/m2/h)
40
30
20
10
0
***
***
1st visit
2nd visit
3rd visit
TEWL (g/m2/h)
50
45
40
35
30
25
20
15
10
5
0
1st visit
2nd visit
3rd visit

Fig. 4

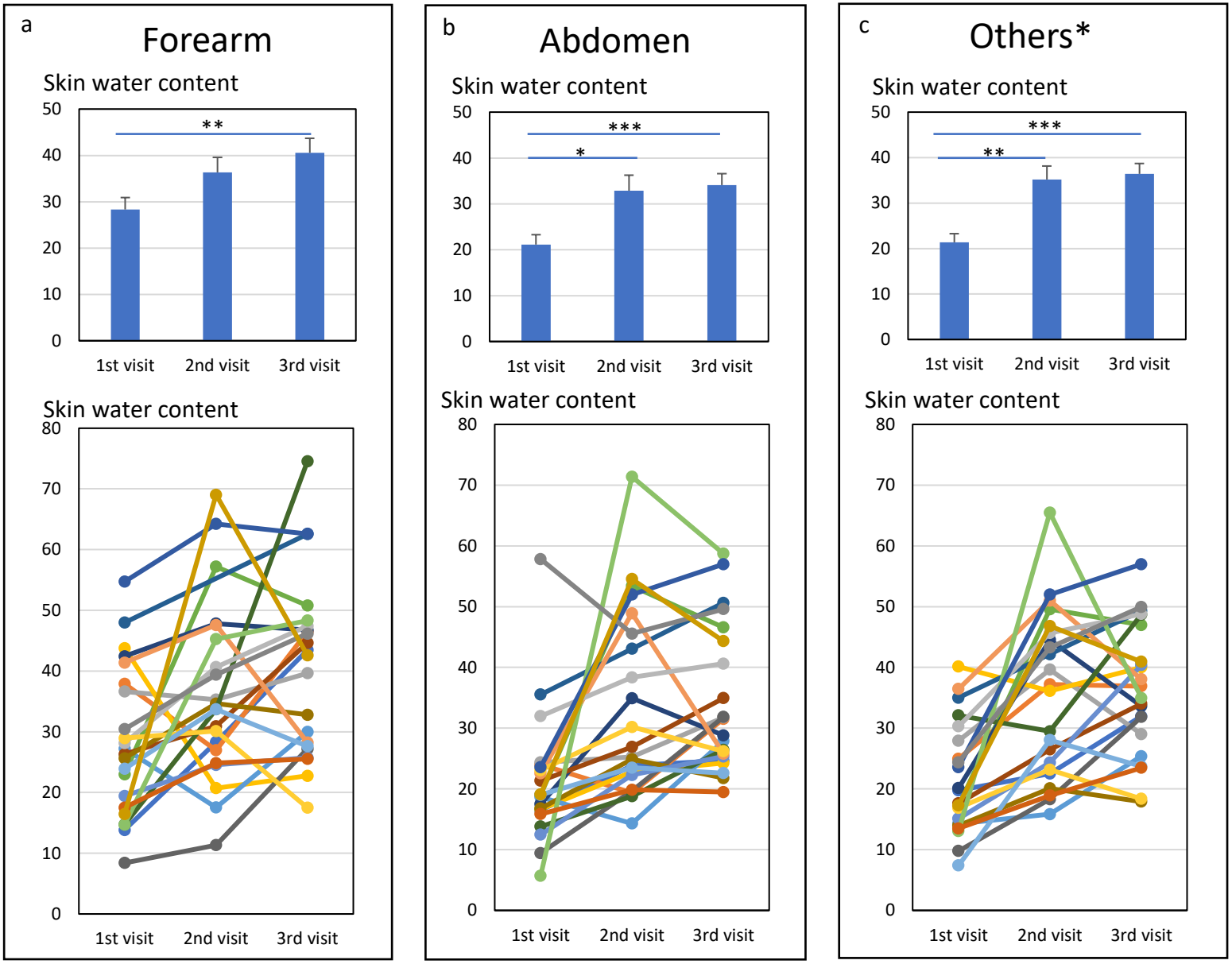
a
Forearm
Skin water content
50
40
30
20
10
0
**
1st visit
2nd visit
3rd visit
Skin water content
80
70
60
50
40
30
20
10
0
1st visit
2nd visit
3rd visit
b
Abdomen
Skin water content
50
40
30
20
10
0
***
*
1st visit
2nd visit
3rd visit
Skin water content
80
70
60
50
40
30
20
10
0
1st visit
2nd visit
3rd visit
c
Others*
Skin water content
50
40
30
20
10
0
***
**
1st visit
2nd visit
3rd visit
Skin water content
80
70
60
50
40
30
20
10
0
1st visit
2nd visit
3rd visit

Fig. 5

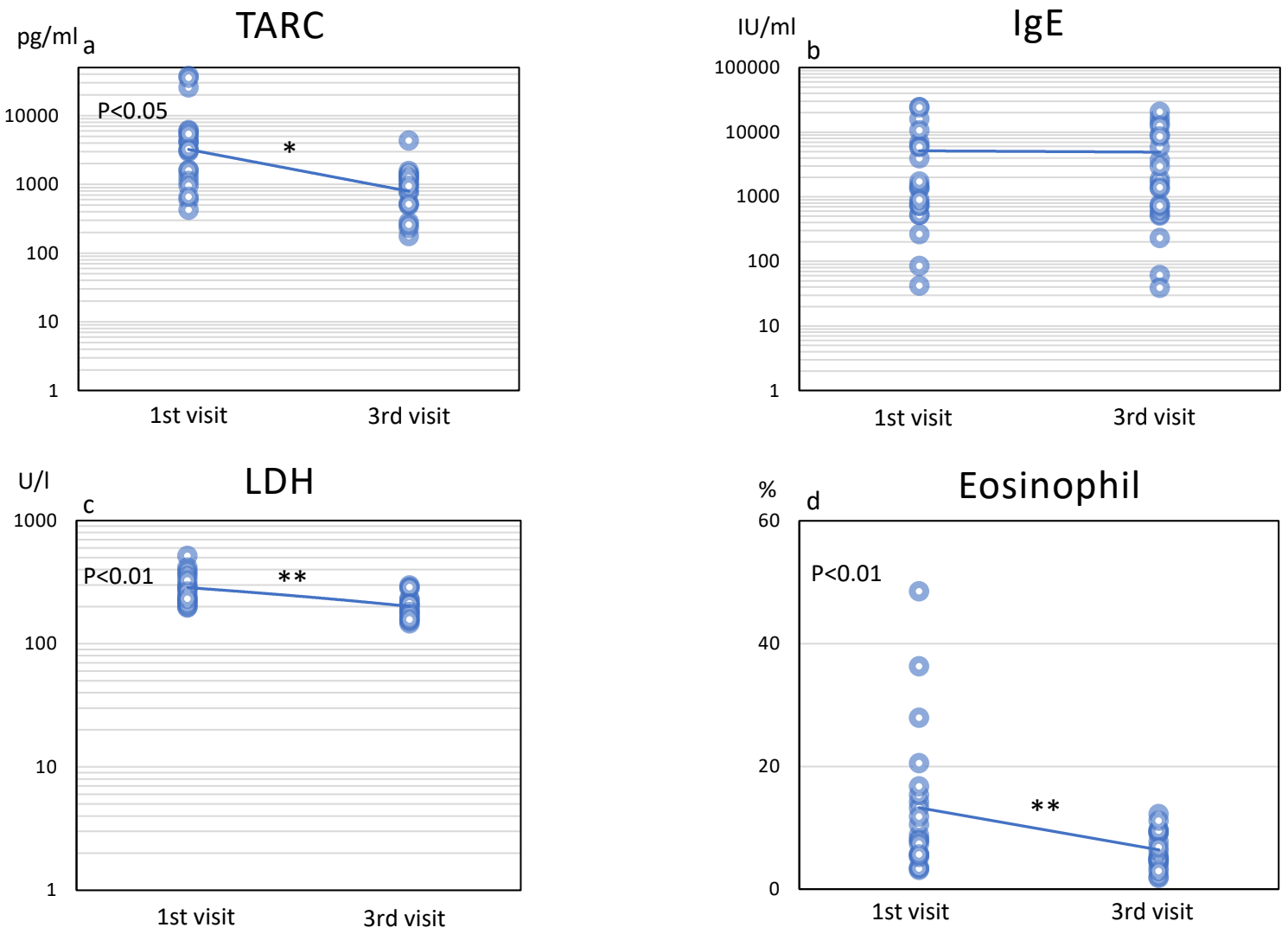
pg/ml
a
TARC
10000
1000
100
10
1
P<0.05
*
1st visit
3rd visit
IU/ml
b
IgE
100000
10000
1000
100
10
1
1st visit
3rd visit
U/l
c
LDH
1000
100
10
1
P<0.01
**
1st visit
3rd visit
%
d
Eosinophil
60
40
20
0
P<0.01
**
1st visit
3rd visit

Fig. 6

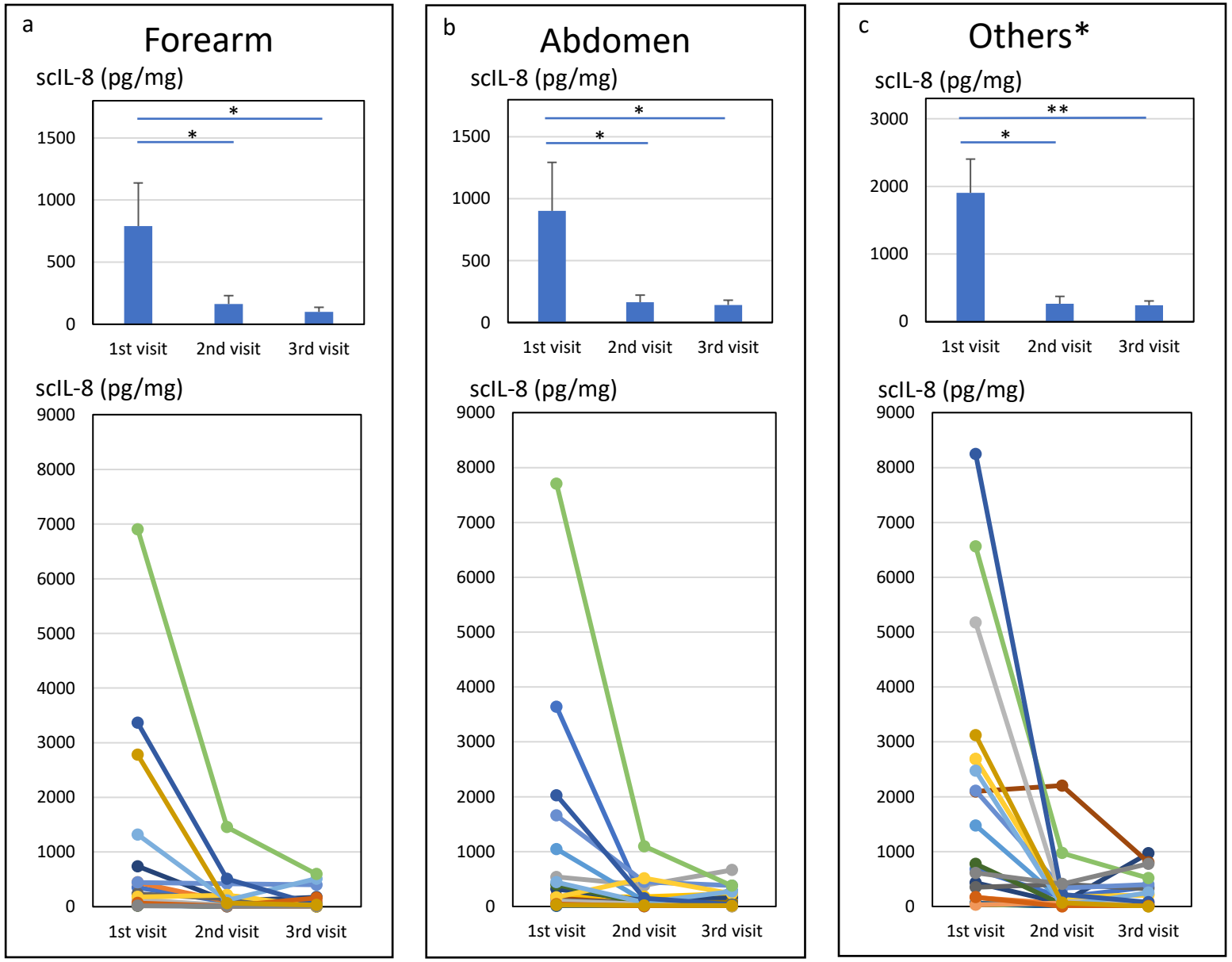
a
Forearm
scIL-8 (pg/mg)
1500
1000
500
0
*
*
1st visit
2nd visit
3rd visit
scIL-8 (pg/mg)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1st visit
2nd visit
3rd visit
b
Abdomen
scIL-8 (pg/mg)
1500
1000
500
0
*
*
1st visit
2nd visit
3rd visit
scIL-8 (pg/mg)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1st visit
2nd visit
3rd visit
c
Others*
scIL-8 (pg/mg)
3000
2000
1000
0
**
*
1st visit
2nd visit
3rd visit
scIL-8 (pg/mg)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1st visit
2nd visit
3rd visit

Fig. 7

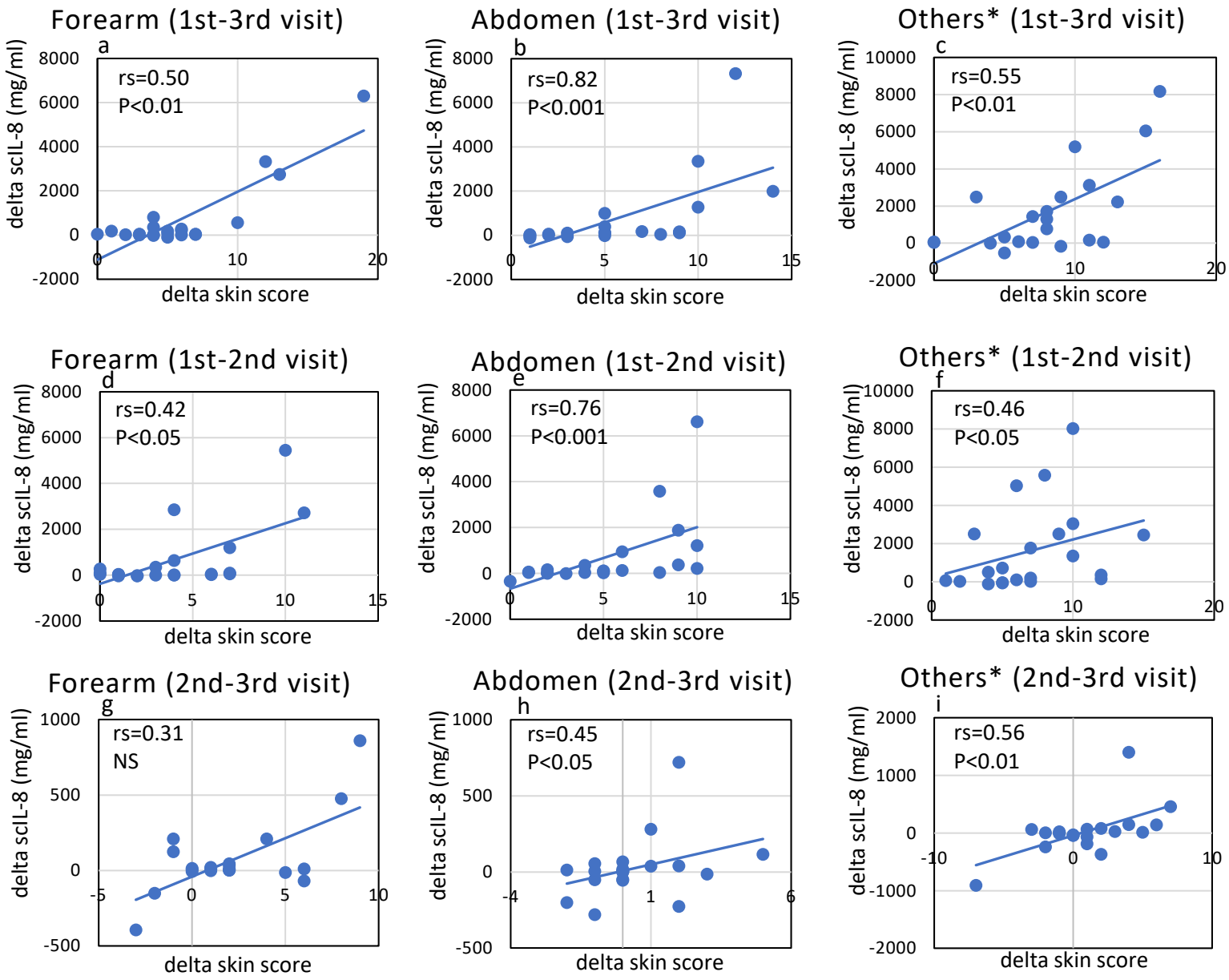
Forearm (1st-3rd visit)
a
rs=0.50
P<0.01
delta scIL-8 (mg/ml)
delta skin score
Abdomen (1st-3rd visit)
b
rs=0.82
P<0.001
delta scIL-8 (mg/ml)
delta skin score
Others* (1st-3rd visit)
c
rs=0.55
P<0.01
delta scIL-8 (mg/ml)
delta skin score
Forearm (1st-2nd visit)
d
rs=0.42
P<0.05
delta scIL-8 (mg/ml)
delta skin score
Abdomen (1st-2nd visit)
e
rs=0.76
P<0.001
delta scIL-8 (mg/ml)
delta skin score
Others* (1st-2nd visit)
f
rs=0.46
P<0.05
delta scIL-8 (mg/ml)
delta skin score
Forearm (2nd-3rd visit)
g
rs=0.31
NS
delta scIL-8 (mg/ml)
delta skin score
Abdomen (2nd-3rd visit)
h
rs=0.45
P<0.05
delta scIL-8 (mg/ml)
delta skin score
Others* (2nd-3rd visit)
i
rs=0.56
P<0.01
delta scIL-8 (mg/ml)
delta skin score

Fig. 8

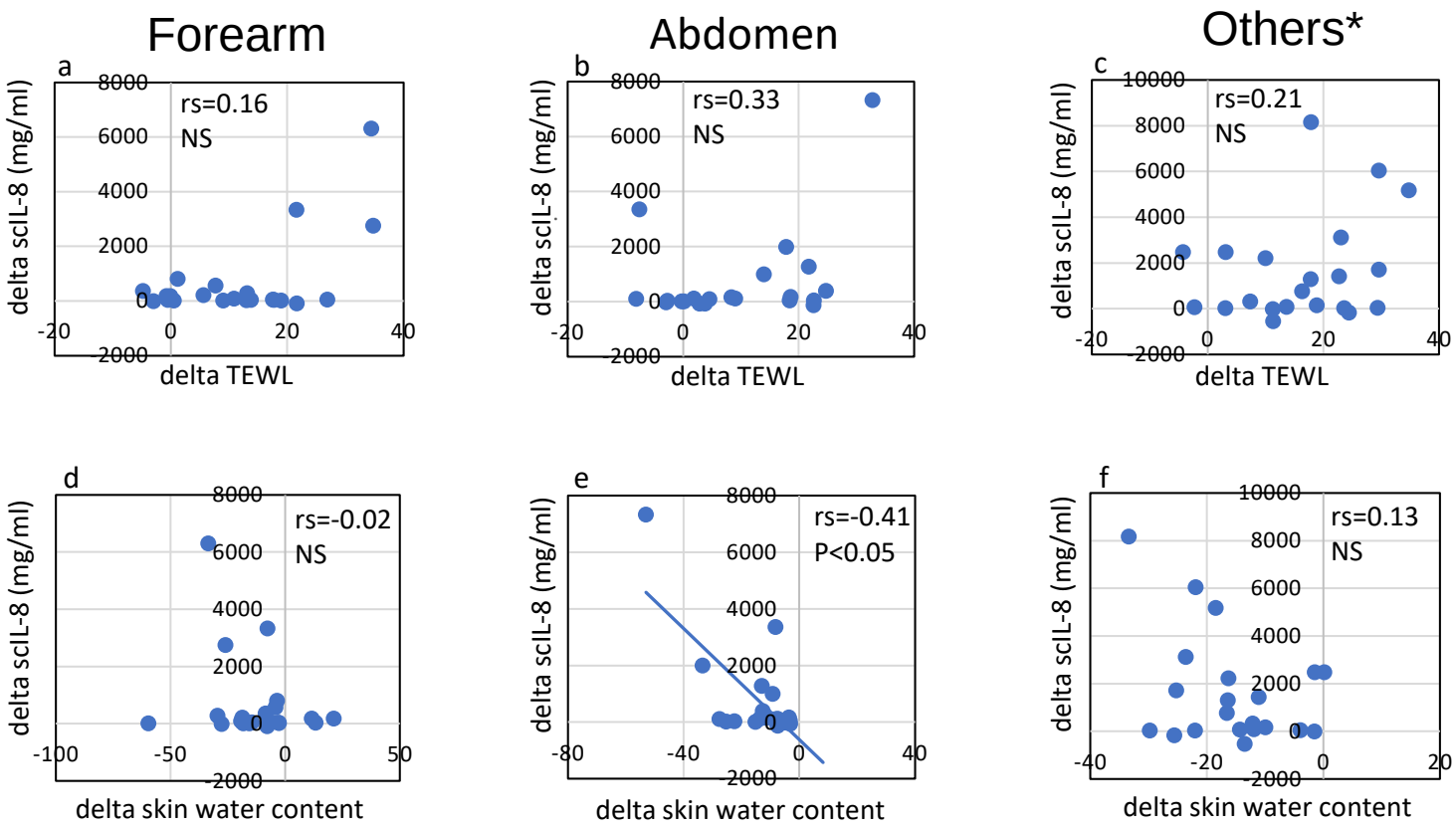
Forearm
Abdomen
Others*
a
b
c
d
e
f
delta scIL-8 (mg/ml)
delta TEWL
delta skin water content
rs=0.16
NS
rs=0.33
NS
rs=0.21
NS
rs=-0.02
NS
rs=-0.41
P<0.05
rs=0.13
NS

Fig. 9

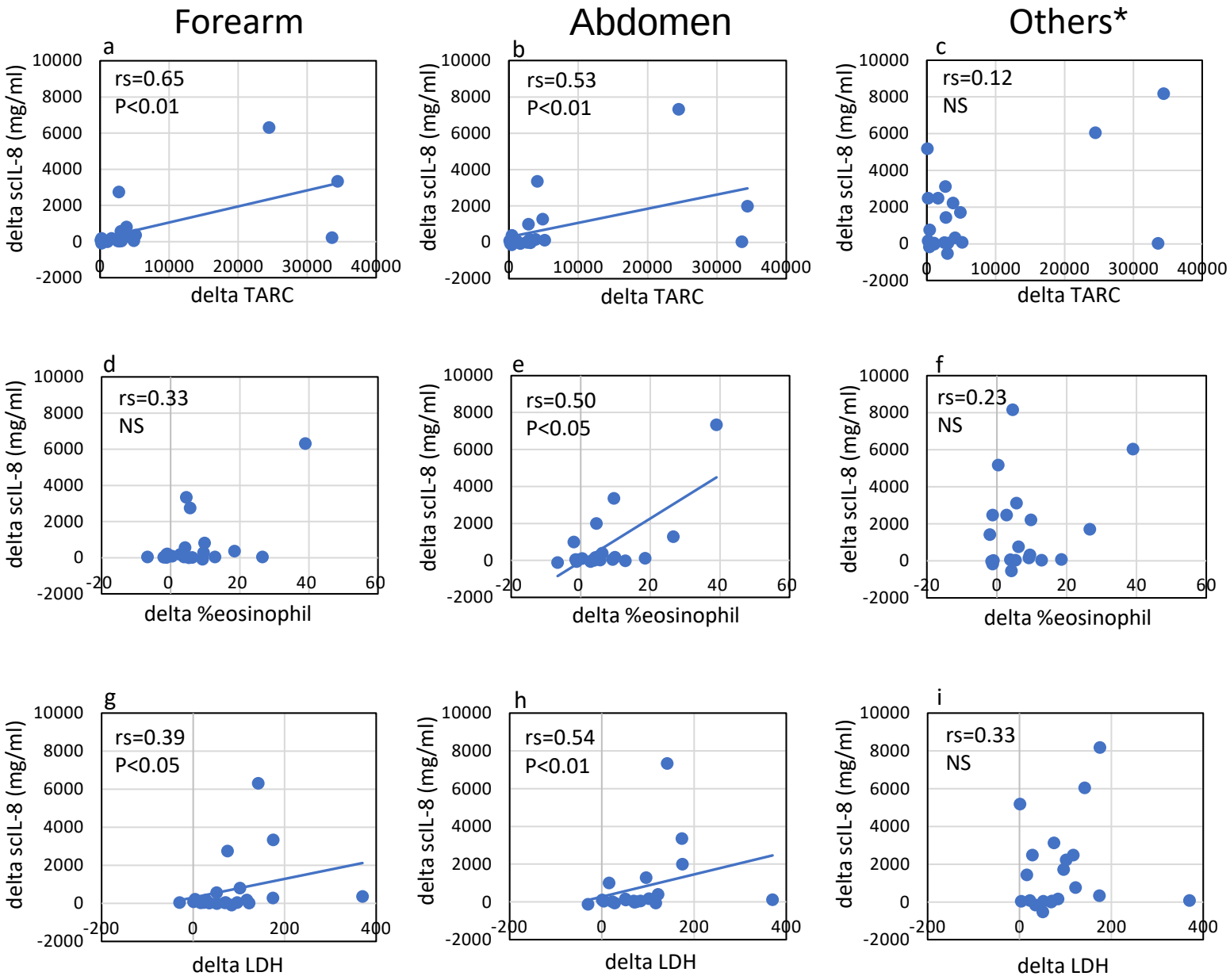
Forearm
Abdomen
Others*
a
b
c
rs=0.65
P<0.01
rs=0.53
P<0.01
rs=0.12
NS
delta scIL-8 (mg/ml)
delta TARC
d
e
f
rs=0.33
NS
rs=0.50
P<0.05
rs=0.23
NS
delta %eosinophil
g
h
i
rs=0.39
P<0.05
rs=0.54
P<0.01
rs=0.33
NS
delta LDH

Fig. 10

Table 1. Background of patients

Panelist	Age	Sex	Laboratory data					A	B						C		SCORAD
			TARC	IgE	LDH	WBC	Eosinophil	Affected	Erythema	Edema	Exudation	Scratch	Licheni-	Dryness	Pruritus	Sleepless	
			pg/ml	IU/ml	U/l	×1000/μL	%	lesions %					fication				
1	31	M	5206	3906	374	7.9	14.2	50	2	2	0	2	2	2	7	3	55
2	16	F	5920	1341	516	10.2	27.9	30	1	1	0	2	2	1	9	8	48
3	34	F	1622	24271	200	10.5	5.5	50	1	1	0	2	1	1	5	0	36
4	44	M	2979	15955	415	6.6	8.0	42	2	1	1	1	3	3	4	2	53
5	25	M	4217	84.8	247	6.5	3.1	80	2	2	0	1	1	2	8	3	55
6	14	F	1324	803	215	8.8	8.8	40	1	0	0	1	1	2	8	8	42
7	38	F	4363	6857	236	3.7	13.2	70	2	2	2	2	3	3	6	5	74
8	21	M	n/a	n/a	n/a	n/a	n/a	30	1	1	0	0	0	1	3	0	20
9	28	F	37910	24200	286	6.0	3.5	32	3	2	0	3	2	3	7	5	64
10	37	F	4016	6014	280	7.7	20.5	96	3	3	0	3	2	3	9	4	81
11	26	F	3091	524	228	7.0	5.7	15	1	0	0	0	0	1	8	4	22
12	28	M	593	519	301	5.8	10.5	62	1	1	1	1	1	2	3	2	42
13	14	M	6123	728	286	5.7	36.3	40	2	2	0	2	2	2	7	0	50
14	24	F	1582	1351	226	6.7	3.3	43	2	3	1	1	1	2	8	5	57
15	38	F	1120	41.9	213	5.2	5.2	29	2	1	2	1	1	2	4	0	41
16	17	M	954	1499	197	8.1	8.0	24	1	0	0	0	1	2	9	7	35
17	31	M	5377	10587	390	9.5	11.8	90	2	2	1	2	2	2	7	6	70
18	19	M	25720	1708	354	10.7	48.5	86	3	2	3	3	1	3	7	8	85
19	40	M	35330	720	327	6.7	7.4	68	3	2	2	2	2	2	7	5	71
20	20	M	424	264	265	7.4	15.3	80	2	1	0	2	2	2	0	10	58
21	33	F	657	5873	208	7.3	5.6	20	2	2	0	1	2	2	3	0	39
22	49	F	3225	890	232	5.1	16.7	50	3	3	0	2	2	2	3.5	8	64
Mean	28.5		7226	5149	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			2367	1600	18	0.4	2.5	5.0	0.2	0.2	0.2	0.2	0.2	0.1	0.5	0.7	3.7

All patients' serum laboratory data and SCORAD indexes were obtained before starting topical corticosteroid treatment.
SCORAD, Scoring Atopic Dermatitis; n/a, 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.

Table 2. Total skin score, transepidermal water loss (TEWL), skin water content, and stratum corneum interleukin-8 (scIL-8) concentration over the test period

scIL-8, pg/mg	1st visit	2nd visit	3rd visit
Forearm	790.3 ± 348.0	162.9 ± 67.9	99.6 ± 37.0
Abdomen	902.1 ± 391.4	165.0 ± 56.6	142.0 ± 38.4
Others*	1904.8 ± 499.6	267.1 ± 108.3	242.8 ± 65.1
Total skin score	**1st visit**	**2nd visit**	**3rd visit**
Forearm	7.8 ± 1.0	4.0 ± 0.7	2.0 ± 0.4
Abdomen	7.3 ± 0.9	1.9 ± 0.4	1.5 ± 0.3
Others*	10.4 ± 0.9	3.5 ± 0.6	2.5 ± 0.5
TEWS, g/m2/h	**1st visit**	**2nd visit**	**3rd visit**
Forearm	19.0 ± 2.7	9.9 ± 1.5	7.2 ± 1.2
Abdomen	19.5 ± 2.5	11.4 ± 2.4	9.9 ± 1.8
Others*	28.5 ± 1.8	14.9 ± 2.1	12.2 ± 1.7
Skin water content	**1st visit**	**2nd visit**	**3rd visit**
Forearm	28.3 ± 2.6	36.4 ± 3.2	40.6 ± 3.1
Abdomen	21.1 ± 2.2	32.9 ± 3.4	34.1 ± 2.5
Others*	21.4 ± 1.9	35.2 ± 3.0	36.4 ± 2.3

Data are expressed as the mean ± standard error of the mean.