

Content of Vascular Endothelial Growth Factor in Stratum Corneum Well Correlates to Local Severity of Acute Inflammation in Patients with Atopic Dermatitis

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Key Words

Atopic dermatitis • Eczema • Stratum corneum • Tape stripping • Vascular endothelial growth factor

Abstract

Background: Atopic dermatitis (AD) is an inflammatory skin disease, characterized by existence of both acute and chronic eczema. Various markers are used to clinically evaluate the severity of AD as a whole. However, little is known regarding markers that can efficiently indicate the severity of a localized lesion. Vascular endothelial growth factor (VEGF), a potent activator of vascular permeability, is known to be increased in AD lesions. In order to establish whether the VEGF content in the stratum corneum (scVEGF) can be used as a marker to evaluate severity of AD lesions, we evaluated the association between scVEGF and symptom scores of localized lesions. **Methods:** Fifty patients with AD and 12 healthy subjects were enrolled. Skin lesions were evaluated and transepidermal water loss and skin water content of the lesions were measured. Stratum corneum samples were collected from the skin of back, neck and arm by the tape stripping technique. The scVEGF were evaluated using a VEGF-specific ELISA method after extracting protein from the scales. **Results:** The scVEGF levels were significantly higher

in patients with AD than in healthy controls. Moreover, the scVEGF levels highly correlated with the manifestation scores of erythema and edema/papulation, and weakly correlated with the scores of excoriation, xerosis and itch. They also correlated significantly with transepidermal water loss and skin water content. **Conclusions:** The scVEGF levels correlated well with the severity of clinical conditions, especially erythema and edema/papulation. scVEGF level is considered to be a useful marker to evaluate acute inflammatory conditions in individual AD lesions.

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Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by intense pruritus with a mixed condition of acute and chronic eczema. Various factors contribute to the pathogenesis and development of AD. These factors include immunological abnormalities as well as physiological and biochemical barrier dysfunctions of the skin. In acute AD, skin lesions are erythematous papules associated with excoriation and serous exudation, with no tissue remodeling. In chronic AD, skin lesions have undergone tissue remodeling

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caused by chronic inflammation. Th2-type immune response is dominant in the acute phase of skin lesions, although Th1-type immune response contributes to the chronic phase of the lesions [1, 2].

Vascular endothelial growth factor (VEGF) is a cytokine that potently promotes survival, migration and proliferation of endothelial cells. It has been reported that the expression of VEGF is upregulated in the lesional epidermis of inflammatory dermatoses such as psoriasis and contact dermatitis [3, 4]. We have shown that VEGF is largely overproduced in the epidermis of AD lesions and this overproduction results in vessel dilatation and hyperpermeability [5]. Since prolonged dermal capillary dilatation is seen in the typical lesions of AD, it is likely that VEGF also participates in the pathogenesis of AD [6].

In dermatological clinics, no means of evaluating the severity of individual lesions in AD have been established. To establish such means, we previously reported a method that involved the microscopic measurement of fluorescence which reflects thymus- and activation-regulated chemokine (TARC) levels in tape-stripped skin samples [7]. However, this technique requires a fluorescent microscope and, as a result, is not easily done. In this study, we measured the content of VEGF in tape-stripped stratum corneum samples (scVEGF) obtained from lesional skin in patients with AD by using ELISA method and evaluated the correlation between the scVEGF and clinical severity scores of localized skin lesions.

Subjects and Methods

Subjects

Fifty patients with AD (11–58 years, average age 28.3 years) who fulfilled the diagnostic criteria for AD established by the Japanese Dermatological Association were enrolled in the study [8]. Of the 50 patients, 29 were men (14–58 years, mean age 26.8 years) and 21 were women (11–55 years, mean age 30.2 years). A total of 12 healthy subjects (22–50 years, mean age 28.8 years) consisting of 8 men (24–32 years, mean age 26.9 years) and 4 women (22–50 years, mean age 31.6 years) formed the control group. The inclusion criteria for healthy subjects were as follows: (1) those without any inflammatory skin diseases, (2) those whose physical findings were normal and (3) those with no past history or family history related to allergies. Prior to initiating this study, the study and methodology to be used were fully explained to each subject or his/her guardian and written informed consent was obtained. This study was approved by the ethical committee of Shimane University, Faculty of Medicine (approval No. 459).

Clinical Severity of AD

The clinical severity of AD was evaluated and defined as slight, mild, moderate and severe according to the diagnostic criteria for

AD set by the Japanese Dermatological Association [8]. The 50 patients with AD were classified in the following manner: 7 patients had severe AD, 11 patients had moderate AD, 9 patients had mild AD and 23 patients had slight AD.

The severity scoring of AD (SCORAD) was calculated by the equation $A/5 + 3.5B + C$, where A is the percentage of body area with a skin rash, B is the total score of erythema, edema/papulation, excoriation, oozing/crust and lichenification, each of which was evaluated on a 4-point scale (0 = none; 1 = mild; 2 = moderate; 3 = severe), and C is the sum of the visual analogue scale (a scale ranging from 0 to 10), which was used in the subjective assessment of both itching and sleep loss [9].

Scoring of the Skin Lesions

The manifestation of the lesions associated with AD was evaluated on a 4-point scale (0 = none; 1 = mild; 2 = moderate; 3 = severe) for each parameter of skin symptoms described in SCORAD: erythema, edema/papulation, excoriation, oozing/crust, lichenification and xerosis [9].

Measurement of Transepidermal Water Loss and Skin Water Content

Transepidermal water loss (TEWL) and skin water content (SWC) were measured using Corneometer® CM825 and Tewameter® TM300 MPA5 (Courage + Khazako, Cologne, Germany) in an ordinary air-conditioned room at the clinic.

Laboratory Tests

Among the 50 patients who were enrolled in the study, there were 47 patients with AD (11–58 years, mean age 27.8 years) that consented to the blood tests. Of these, 27 were men (14–58 years, mean age 26.9 years) and 20 were women (11–55 years, mean age 28.9 years). Serum TARC level, serum total IgE level, serum LDH level and peripheral blood eosinophil count were measured by laboratory tests. The results of the 47 patients were as follows: the serum TARC level was $3,648 \pm 7,751$ pg/ml, while the serum IgE level was $3,391 \pm 6,161$ IU/ml and the serum LDH level was 233 ± 80 IU/l. Finally, the eosinophil count was 619 ± 648 cells/ml.

Measurement of the scVEGF

The stratum corneum was collected, using tape stripping technique, from skin lesions of the forearm, neck and back [7]. Plastic tape (24 × 35 mm, Serotape®; Nichiban, Tokyo, Japan) was applied and removed 5 times to the skin lesions and stored at –20°C until use. The tapes were cut finely and immersed in 5 ml hexane. After collecting horny layer by centrifugation, proteins were extracted in 1 ml of extraction buffer (contains 0.1 M Tris-HCl, pH 8.0, and 0.5% Triton X-100) under ultrasound sonication (Branson Sonifer 450; Emerson Japan, Atsugi, Japan) for 3 min. The supernatant was purified using a 4-mm filter (Millex®; Millipore, Tokyo, Japan) and then subjected to centrifugation at 15,000 rpm for 15 min at 4°C. The purified supernatant was then used as extract samples of stratum corneum. The amount of VEGF in the extracts was measured using an enzyme-linked immunosorbent assay (ELISA) kit specific for VEGF (R&D Systems, Minneapolis, Minn., USA) and SuperSignal® ELISA Femto Maximum Sensitivity Substrate (Thermo Scientific, Rockford, Ill., USA). Total protein levels of the samples were measured by DC protein assay (Bio-Rad, Hercules, Calif., USA).

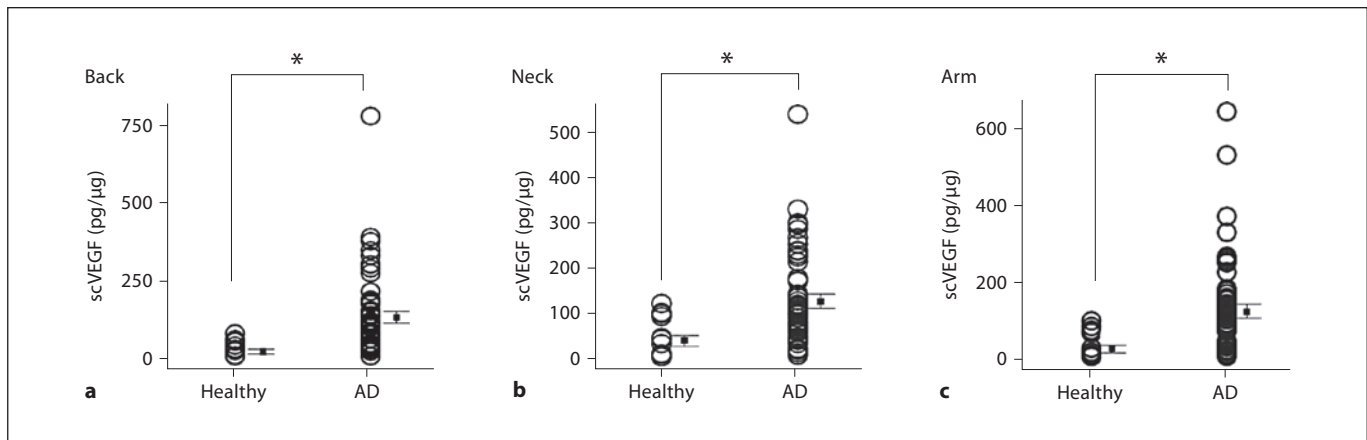


Fig. 1. Correlation between the scVEGF and clinical severity. The scVEGF of the back (a), neck (b) and arm (c) (n = 50) were significantly higher in patients with AD than in healthy subjects (n = 12). * p < 0.01.

Table 1. Characteristics of AD patients

Severity	n	SCORAD	IgE, IU/ml	LDH, IU/l	TARC, pg/ml	Eosinophil, cells/μl
Severe	7	61.5 ± 16.9	7,731 ± 11,046	317 ± 86	11,634 ± 13,154	1,396 ± 818
Moderate	11	45.7 ± 12.9	6,855 ± 5,654	268 ± 38	3,923 ± 4,613	923 ± 700
Mild	9	23.5 ± 7.9	1,301 ± 2,327	192 ± 54	528 ± 251	273 ± 180
Slight	23	7.1 ± 5.3	801 ± 1,147	198 ± 68	1,710 ± 5,685	312 ± 216
Total	50	26.2 ± 3.3	3,391 ± 6,161	233 ± 80	3,648 ± 7,751	619 ± 648

Values are means ± SEM. Severity of AD was determined by the criteria set by the Japanese Dermatological Association [8]; SCORAD was determined by the criteria reported previously [9].

Data Analysis

For statistical analysis. Mann-Whitney's U test was used for comparison between the 2 groups and Spearman's rank correlation test was used for correlation. Results are expressed as means ± SEM. The results were considered to be significant or correlated at p < 0.01 or p < 0.05.

Results

Characteristics of AD Patients

Characteristics of the enrolled patients with AD are shown in table 1. SCORAD indexes of severe, moderate, mild and slight groups were 61.5 ± 6.4, 45.7 ± 3.9, 23.5 ± 2.6 and 7.7 ± 1.1, respectively. The clinical severity of the disease was evaluated using the diagnostic criteria for AD set by the Japanese Dermatological Association and was well correlated with the SCORAD indexes.

Correlation between scVEGF and Clinical Severity in AD Patients

The scVEGF levels in AD patients and healthy controls are shown in figure 1. The scVEGF in the back, the neck and the arm of AD patients were 138.6 ± 19.5, 130.4 ± 14.8 and 129.4 ± 17.9 pg/μg, respectively. The same areas produced the following data values in healthy controls: 29.3 ± 6.9, 43.4 ± 12.7 and 29.9 ± 10.2 pg/μg, respectively.

A significant correlation was seen between the scVEGF and the SCORAD index in the back and arm but not in the neck (fig. 2).

Correlation between scVEGF and Each Parameter of Skin Symptoms

The correlation of the scVEGF was assessed using the manifestation scores of skin lesions (total score, erythe-

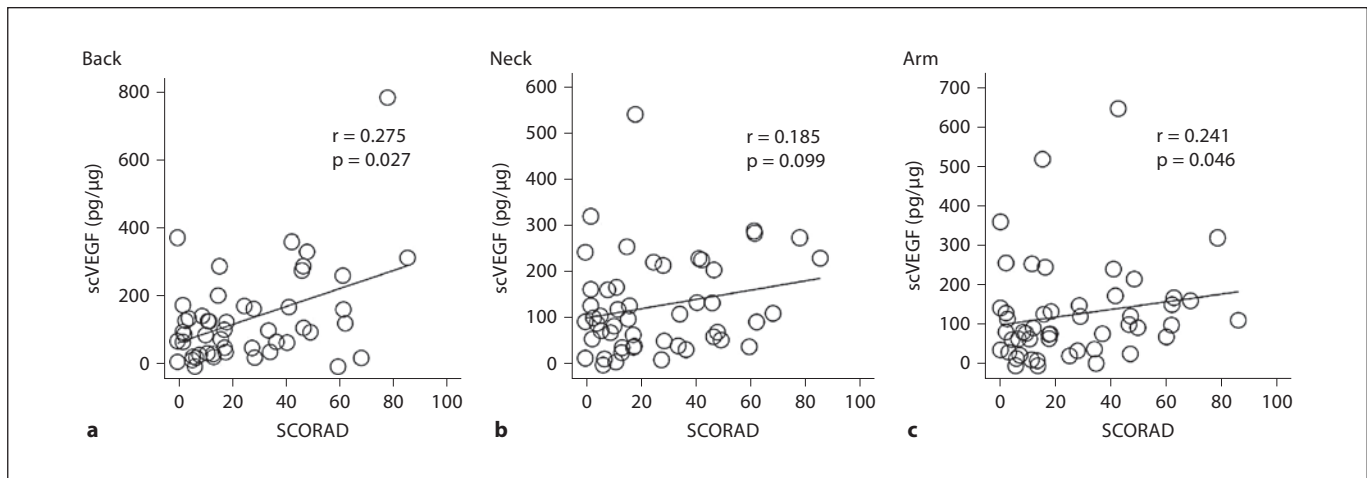


Fig. 2. Correlation between the scVEGF and SCORAD. The scVEGF of the back (a), neck (b) and arm (c) in patients with AD are shown. The scVEGF of the back and arm correlates with clinical scores (SCORAD) of AD. * Significantly different from cases of mild clinical condition, $p < 0.05$.

ma, edema/papulation, excoriation, xerosis, itch, lichenification and oozing/crust scores) in the affected skin of the back in patients with AD. The scVEGF correlated significantly with the total score of eruptions (fig. 3a). Moreover, the scVEGF correlated significantly with the erythema (fig. 3b), edema/papulation (fig. 3c), excoriation (fig. 3f), xerosis (fig. 3g) and itch scores (fig. 3h). The correlation was not significant in regards to the lichenification (fig. 3d) and oozing/crust scores (fig. 3e). Similar correlation was also observed in the neck and arm of patients with AD (data not shown).

Correlation between scVEGF and Skin Barrier Parameters

Correlation between the scVEGF of the skin (back, neck and arm) and skin barrier parameters (TEWL and SWC) in the patients are shown in figure 4. The scVEGF correlated well to TEWL in the back. However, there was no significant level of correlation between scVEGF and SWC in all locations.

Correlation between scVEGF and Laboratory Data

The scVEGF content of the back of the patients exhibiting symptoms of AD was compared with serum TARC level, serum total IgE level, serum LDH level and blood eosinophil count (fig. 5). The scVEGF content positively correlated with the serum TARC level, the serum LDH level, and the eosinophil count. However, no significant correlation was observed between the scVEGF content

and the serum IgE level. The serum LDH and eosinophil count exhibited a significant correlation with scVEGF. In contrast, the serum total IgE did not significantly correlate with scVEGF.

Discussion

In this study, we determined scVEGF content in the patients with AD using the tape-stripping method and found that the scVEGF content highly correlated with the severity scores of erythema and edema/papulation in the localized skin lesions (fig. 3). Considering that such symptoms are of acute lesions of AD, our results indicate that the scVEGF content may be used as an indicator of the acute inflammatory condition of skin lesions in the patients with AD. Also, these results provide evidence supporting the idea that VEGF produced in the epidermis plays an important role in the pathogenesis of the AD, especially in the prolonged capillary dilatation and edema that is characteristic of AD. However, this growth factor has a lesser role in the chronic phase of AD, as do barrier dysfunctions of the skin.

The results are consistent with our previous report [7] that examined levels scTARC in patients with AD. We reported that scTARC level correlated well with erythema, edema/papulation and oozing/crust scores, but not with the symptom scores of lichenification and xerosis. We consider that both scVEGF and scTARC levels are

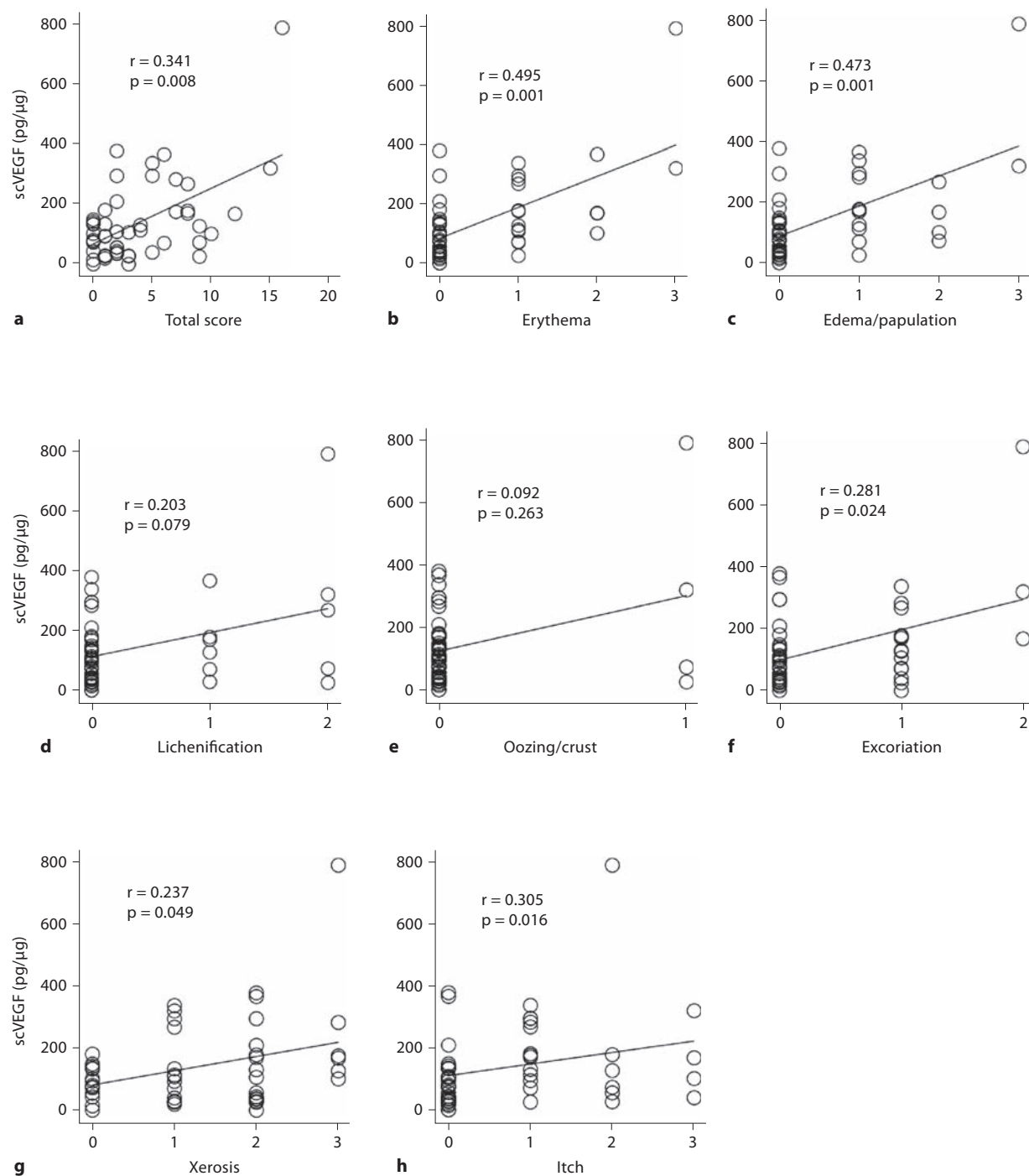


Fig. 3. Correlation between the scVEGF content and manifestation score of skin lesions in the backs of patients with AD. The scVEGF content in the backs of patients with AD and severity score of skin symptoms total score (a), erythema (b), edema/papulation (c), lichenification (d), oozing/crust (e), excoriation (f),

xerosis (g) and itch (h) are shown. Significant correlation was found between the scVEGF and total scores, erythema, edema/papulation, excoriation and itch, but not with oozing/crust and lichenification.

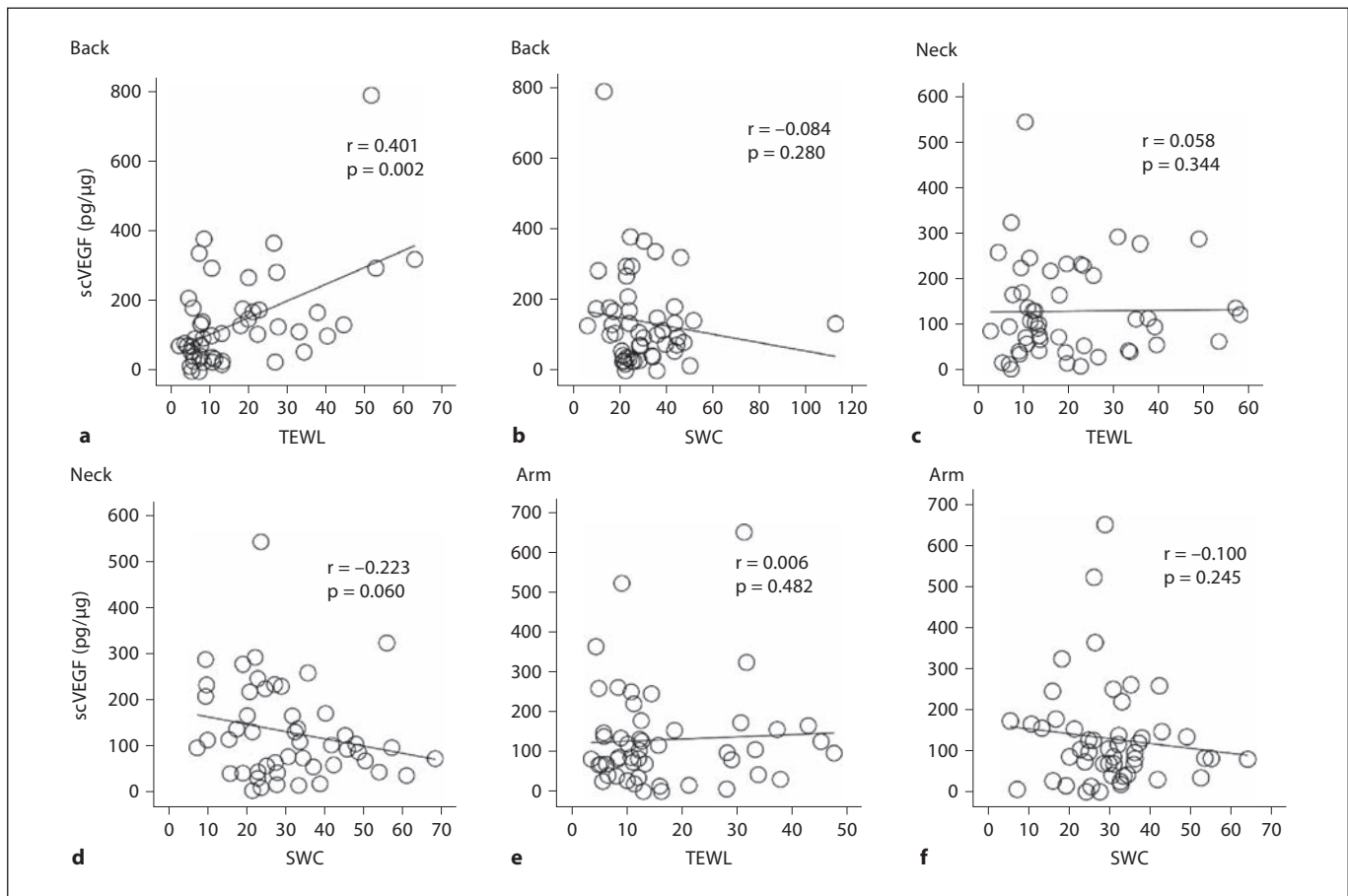


Fig. 4. Correlation between the scVEGF and skin barrier parameters (TEWL, SWC). The scVEGF content in the back between TEWL (**a**) and SWC (**b**), the scVEGF content in the neck between TEWL (**c**) and SWC (**d**), the scVEGF content in the arm between TEWL (**e**) and SWC (**f**) are shown. The scVEGF content in the back correlates with TEWL.

useful in the evaluation of lesions of AD. However, the technical procedure necessary to evaluate the scTARC levels requires fluorescent microscopy due to the minute amount of scTARC present, and as a result, the method is relatively complex and difficult. On the other hand, our method of detecting scVEGF is easy to perform and it is possible to be completed in a short amount of time. The scVEGF levels of the back were found to be a good marker of the clinical severity of the AD, because the scVEGF level was significantly correlated with clinical severity (fig. 1), SCORAD (fig. 2), serum LDH, serum TARC and blood eosinophil count in the patients with AD (fig. 5).

A number of growth factors with proven or potential roles in vascular development and remodeling have been identified including some that are associated with

various disease conditions [10]. VEGF must be included in the growing family of keratinocyte-derived pro-inflammatory cytokines that must be examined in order to determine if they play a pathogenic role in cutaneous inflammation [11]. The pro-inflammatory effects of VEGF may be observed in the colonic endothelium [12]. Moreover, VEGF stimulation of the angiogenic process could contribute to the sustenance of chronic inflammation through changes in microvascular permeability, provisional matrix deposition, remodeling, altered vascular tone and increased blood flow as well as increased vascular surface area available for leukocyte-endothelial cell interactions [13]. To investigate the role of VEGF in the lesion of AD, solid distribution of the cytokine is needed. We do not have these data and this is our future subject.

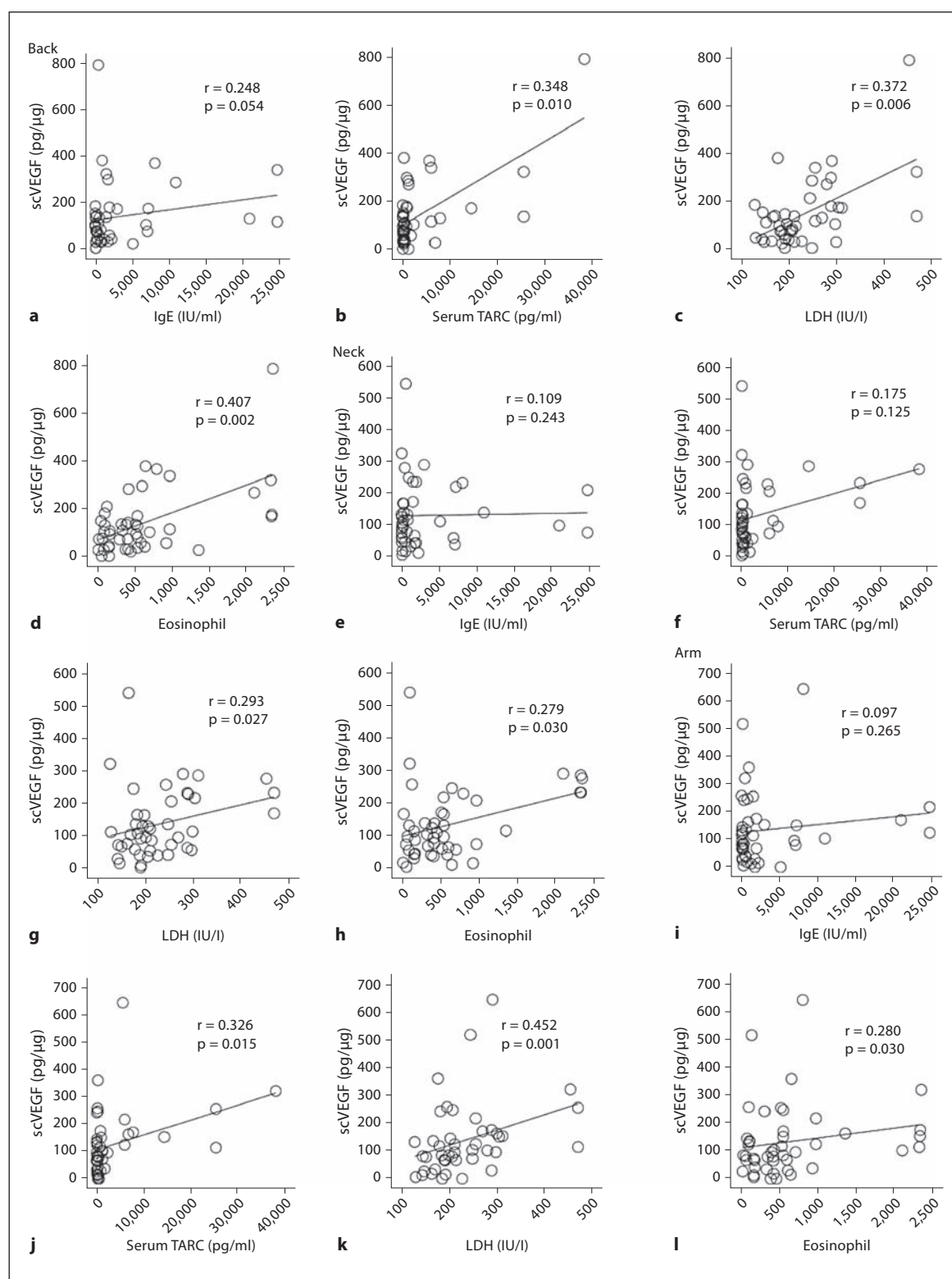


Fig. 5. Correlation between the scVEGF and serum IgE level, serum TARC level, serum LDH level and blood eosinophil count. The scVEGF content in the back between serum IgE (**a**), serum TARC (**b**), serum LDH (**c**) and eosinophil count (**d**). scVEGF con-

tent in the neck between serum IgE (**e**), serum TARC (**f**), serum LDH (**g**) and eosinophil count (**h**) as well as scVEGF content in the arm between serum IgE (**i**), serum TARC (**j**), serum LDH (**k**) and eosinophil count (**l**) are shown.

Our present results revealed that the scVEGF is well correlated with the severity of individual lesions, especially in the areas of erythema and edema/papulation. Moreover, our ELISA-based measurement of scVEGF in tape-stripped samples is useful in the evaluation of acute AD lesions.

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