

## Report

# Expression of vascular endothelial growth factor and other cytokines in atopic dermatitis, and correlation with clinical features

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## Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by intense pruritus and epidermal barrier dysfunction that culminates in dry skin, defects in the innate and acquired immune system, and IgE-mediated sensitization to food and aeroallergens. It is well known that AD results from an interaction between genetic and environmental factors; however, the pathogenesis of AD needs further clarification.<sup>1</sup> The initiation and maintenance of skin inflammation and pruritus directly involves a number of cytokines and chemokines produced by lymphocytes and other cells that participate in the pathogenesis of AD, including keratinocytes, monocytes, mast cells, Langerhans cells, macrophages, and eosinophils. Biopsies taken from typical atopic dermatitis skin lesions show signs of inflammatory cell infiltration, edema, fibrosis, and angiogenesis.<sup>2</sup> Studies performed on an AD mouse model have indicated that angiogenesis

## Abstract

**Background** Vascular endothelial growth factor (VEGF) was found increased in the stratum corneum of patients with atopic dermatitis (AD). However, its potential pathogenic role(s) in AD needs further clarification.

**Objective** The aim of this study was to determine whether VEGF serum levels correlate with other selected cytokine levels and features of AD.

**Methods** VEGF and other cytokine levels were measured in 83 patients with AD and in a control group and then correlated with clinical and laboratory parameters of AD.

**Results** The mean serum concentrations of VEGF and tumor necrosis factor  $\alpha$  were significantly higher in patients with AD than in the control group, whereas the mean interleukin eight serum level was lower. VEGF concentrations correlated with the severity of AD as expressed by SCORAD index and objective SCORAD.

**Conclusion** VEGF could be regarded as a potentially important mediator in the pathogenesis of AD, as VEGF levels correlate somewhat with AD severity.

may be a crucial pathologic feature of AD.<sup>3</sup> Vascular endothelial growth factor (VEGF) is regarded as a potent stimulating factor for angiogenesis and vascular permeability in the skin.<sup>4</sup>

The cellular sources of VEGF are endothelial cells, fibroblasts, smooth muscle cells, and macrophages.<sup>5</sup> This cytokine takes part in many different stages of angiogenesis, including initial vasodilatation, endothelial cell permeability, remodeling of the perivascular matrix by induction of metalloproteinase 1 and plasminogen activators, and in the induction of proliferation and migration of endothelial cells. Moreover, some authors claim that VEGF is regarded as a potentially important mediator in the pathogenesis of contact eczema connected with delayed hypersensitivity.<sup>6</sup>

## Aim of the study

The aim of this study was to determine whether VEGF levels correlate with selected cytokines (interleukin [IL]-6,

IL-8, IL-15, tumor necrosis factor [TNF] $\alpha$ , and CCL5/RANTES) and clinical, immunological, and constitutional features of AD.

## Methods

The study was conducted in Poland and included 83 patients with AD, aged between 18 and 50 years (mean 30.4 years). All included patients had active skin lesions. The diagnosis was based on the classification of Hanifin and Rajka.<sup>7</sup> Characteristics of the examined group are shown in Table 1. The patients with AD used only emollients, topical corticosteroids, and oral antihistamines for skin involvement. Patients with concomitant asthma or allergic rhinitis were allowed to take inhaled and/or nasal steroids. The control group consisted of 25 age- and gender-matched healthy volunteers. The project was approved by the local ethics committee. All participants gave their informed consent before the study.

VEGF blood concentrations were compared to a control group and correlated with clinical parameters listed in Table 1, and serum levels of IL-6, IL-8, IL-15, TNF $\alpha$ , and CCL5 (RANTES), listed in Table 2.

The severity of AD in patients was assessed by the same dermatologist (ZS) using the SCORing Atopic Dermatitis (SCORAD) index and objective SCORAD.<sup>8,9</sup> The distribution of the SCORAD index was achieved using the formula  $A/5 + 7B/2 + C$ . The maximum possible score is 103. Parameter A assesses the extent of the affected area, according to the rule of nines. The intensity of the disease (parameter B) included six symptoms: ery-

thema, edema/papules, excoriation, oozing/crust formation, lichenification, and dryness. Each item has four grades: 0, 1, 2, or 3. In parameter C, subjective symptoms, (i.e., itch and sleeplessness) were calculated and recorded on a scale from 0 to 10 points. The objective SCORAD lacks assessment of itch and sleeplessness; therefore, the maximum is 83 points. The severity of AD was classified as mild <25, moderate 25–50, and severe >50 points according to the SCORAD index.<sup>10</sup> Total IgE concentrations were evaluated using an enzyme-linked immunosorbent assay (ELISA) method with the UniCAP fluorometer (Pharmacia, Uppsala, Sweden). Values above 100 IU/ml were considered as increased.

## Enzyme-linked immunosorbent assays

Venous blood samples were collected into pyrogen-free tubes at the time of clinical assessment. They were allowed to clot at 4 °C for 1 hour and centrifuged at 2000 g for 10 minutes. The samples of sera were stored at –70 °C until used to measure cytokine concentrations by ELISA. The serum was randomly coded, and testing was carried out without knowledge of the clinical status of the subject or any related laboratory data. The ELISAs for IL-6 and IL-8 were done as previously described.<sup>11</sup> The assays for other cytokines were performed using appropriate commercially available ELISA sets, according to the manufacturers' protocols. The ELISA DuoSets (R&D Systems, Minneapolis, MN, USA) were used for the measurement of IL-15, CCL5 (RANTES), and VEGF, while the ELISA Ready-Set-Go (Bioscience, Inc., San Diego, CA, USA) was used to determine TNF $\alpha$  concentrations. All measurements were done in duplicate. The absorption was measured at 492 nm. In each assay, the appropriate recombinant human cytokine or receptor was used to generate the standard curve. Assay sensitivity was 9.0 pg/ml for VEGF; 15 pg/ml for IL-6; 4 pg/ml for IL-8; 15 pg/ml for IL-15; 15 pg/ml for TNF $\alpha$ ; and 15 pg/ml for CCL5 (RANTES).

## Statistical analysis

Statistical analysis was performed using Statistica software version 10.0 (Statsoft, Kraków, Poland). Data estimation with the Shapiro–Wilk test did not confirm a Gaussian distribution. Therefore, the nonparametric Mann–Whitney *U*-test was used to compare interval variables between two groups and Spearman's rank correlation to measure the statistical dependence between two variables, whereas the graphic representation of correlation was shown by scatter plots. Results were displayed as both mean  $\pm$  SD of the mean and median with the interquartile range (25th–75th centile). In all calculations,  $P < 0.05$  was regarded as statistically significant.

**Table 1** Clinical characteristic of patients with AD

|                                |                         |
|--------------------------------|-------------------------|
| Female/male                    | 45/38 (54.2/45.8%)      |
| Duration of AD/years           | 4–49.7, mean 22.1       |
| Personal history of atopy      | 58 (69.9%)              |
| Asthma                         | 18 (21.7%)              |
| Allergic rhinitis              | 35 (42.1%)              |
| Asthma and allergic rhinitis   | 5 (6.0%)                |
| Secondary bacterial infections | 51 (61.4)               |
| SCORAD index                   | 12–95.2, mean 45.6      |
| Extent (% of body area)        | 5–100, mean 42.4        |
| Intensity                      | 2–15, mean 8.6          |
| Subjective symptoms            | 1–20, mean 10.1         |
| SCORAD index severity          |                         |
| Mild < 25                      | 21 (25.3%)              |
| Moderate 25–50                 | 26 (31.3%)              |
| Severe > 50                    | 36 (43.4%)              |
| Objective SCORAD               | 6.0–75.2, mean 35.5     |
| Total IgE (IU/ml)              | 6.8–12 000, mean 1318.8 |
| $\leq 100$                     | 32 (38.6%)              |
| >100                           | 51 (61.4%)              |

AD, atopic dermatitis; SCORAD, SCORing Atopic Dermatitis.

**Table 2** Results of measured cytokines in patients with AD and controls

| Parameter (pg/mL) | AD patients (83)   |                            | Controls (25)      |                            | P value |
|-------------------|--------------------|----------------------------|--------------------|----------------------------|---------|
|                   | Mean $\pm$ SD      | Median (25th-75th centile) | Mean $\pm$ SD      | Median (25th-75th centile) |         |
| VEGF              | 143.4 $\pm$ 177.1  | 106.0 (17.0-184.0)         | 60.7 $\pm$ 76.3    | 45 (0.0-86.0)              | 0.007   |
| IL-6              | 233.0 $\pm$ 528.0  | 0.0 (0.0-217.0)            | 465.9 $\pm$ 775.6  | 0.0 (0.0-774.0)            | NS      |
| IL-8              | 13.7 $\pm$ 52.1    | 0.0 (0.0-0.0)              | 84.5 $\pm$ 215.4   | 0.0 (0.0-72.0)             | 0.01    |
| IL-15             | 0.4 $\pm$ 2.3      | 0.0 (0.0-0.0)              | 0.2 $\pm$ 0.8      | 0.0 (0.0-0.0)              | NS      |
| TNF               | 39.4 $\pm$ 149.9   | 0.0 (0.0-21.0)             | 0.0 $\pm$ 0.0      | 0.0 (0.0-0.0)              | 0.01    |
| CCL5              | 1190.8 $\pm$ 175.5 | 1194.0 (1095.0-1312.0)     | 1227.5 $\pm$ 233.8 | 1278.0 (1202.0-1340.0)     | NS      |

AD, atopic dermatitis; IL, interleukin; NS, not significant; SD, standard deviation; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

## Results

Clinical characteristics of patients with AD are shown in Table 1. Results of measured cytokine levels in patients with AD are shown in Table 2.

The mean serum concentration of VEGF and TNF $\alpha$  was higher in patients with AD ( $P = 0.007$ ;  $P = 0.01$  respectively) than in the control group, whereas the mean IL-8 serum concentration was lower ( $P = 0.01$ ). No statistical differences were found between the mean levels of CCL5 (RANTES), IL-6, and IL-15 in patients with AD versus controls.

VEGF concentration positively correlated with the severity of AD as expressed by the SCORAD index ( $r = 0.31$ ;  $P < 0.05$ ), objective SCORAD ( $r = 0.25$ ;  $P < 0.05$ ) and its components: edema/papules ( $r = 0.32$ ;  $P < 0.05$ ) and exco-riations ( $r = 0.31$ ;  $P < 0.05$ ) (Figs 1 and 2).

No correlations were found with extent (%), erythema, oozing/crusts, lichenification, dryness, subjective SCORAD, itch, and sleeplessness.

No relations were found between serum levels of VEGF and secondary bacterial skin infections, other allergic diseases such as asthma, allergic rhinitis, urticaria, dyspepsia, conjunctivitis, duration of the disease, IgE level, or serum concentrations of IL-6, IL-8, IL-15, TNF $\alpha$ , and CCL5.

Total IgE levels ranged from 6.8 to 12,000 IU/ml, mean 1318.8 IU/ml. In 32 patients (38.6%), the level was increased above 100 IU/ml, but in 51 (61.4%), it was below 100 IU/ml (Table 1). Total IgE level positively correlated with the SCORAD index ( $r = 0.4$ ;  $P < 0.05$ ) and objective SCORAD ( $r = 0.44$ ;  $P < 0.05$ ), whereas it did not correlate with subjective SCORAD.

Serum IL-15 levels correlated with IgE levels ( $r = 0.22$ ;  $P < 0.05$ ) but not with disease severity. No statistical correlations were found between concentrations of IL-6, IL-8, TNF $\alpha$ , and CCL-5, disease severity, and IgE level.

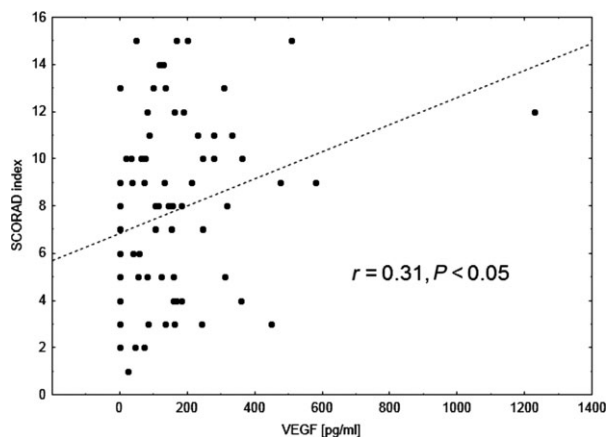


**Figure 1** Correlation between VEGF concentration and SCORAD index. SCORAD, SCORing Atopic Dermatitis; VEGF, vascular endothelial growth factor

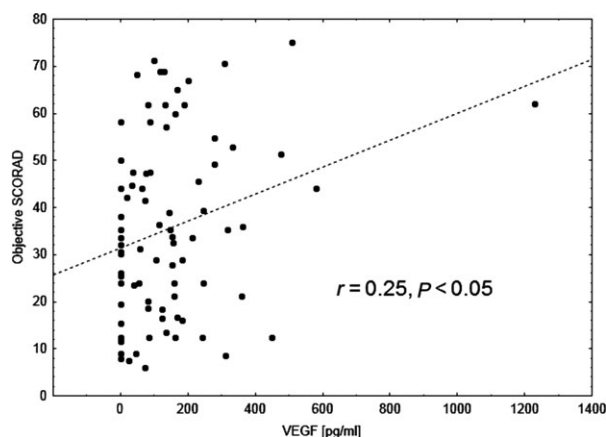


**Figure 2** Correlation between VEGF concentration and objective SCORAD. SCORAD, SCORing Atopic Dermatitis; VEGF, vascular endothelial growth factor

## Discussion

The role of VEGF in patients with AD is not fully understood and remains speculative. Previous studies have assessed VEGF levels in AD skin lesions and in serum. Results have been inconsistent. Some authors found that in VEGF, concentrations are 25 times higher in the stratum corneum of AD lesional skin than in normal stratum corneum.<sup>12</sup> It is also possible that keratinocytes in AD will release greater amounts of VEGF, which in turn could contribute to the subsequent increased blood levels. Amariyasingh *et al.*<sup>13</sup> revealed that the skin level of stratum corneum VEGF was significantly higher in patients with AD than in healthy controls. This cytokine correlated positively with the severity of clinical conditions, in particular erythema and edema/papules. For this reason, authors have concluded that stratum corneum VEGF could serve as a useful clinical marker of acute inflammation in individual patients with AD. Similarly, Chen *et al.*<sup>4</sup> discovered, in a murine model of AD, that epidermal cells were the major producers of VEGF. Moreover, the levels of VEGF in the skin and serum correlated with the intensity of skin inflammation, suggesting possible proinflammatory properties of this protein, though cause and effect are uncertain. However, Koczy-Baron *et al.*<sup>14</sup> in an analysis of 51 patients with AD and 35 controls obtained significantly higher VEGF plasma concentration in patients with AD, but the VEGF levels did not correlate either with the disease severity or the total IgE levels. Our results confirmed that VEGF could be regarded as a potentially important mediator in the pathogenesis of AD; however, without any correlation with IgE levels.

In a very limited study of seven patients with AD, Brockow *et al.*<sup>15</sup> showed that VEGF serum concentrations are only slightly higher than in controls and that the results are not significant. In our larger study of 83 patients with AD and 25 controls, the difference was statistically significant. Such discrepancies could be explained both by differences in laboratory methods and sample size. Some authors have recommended flow cytometry, as a more sensitive diagnostic tool to measure VEGF, instead of the widely used ELISA.<sup>16</sup>

VEGF is regarded not only as a proangiogenic factor, but it also promotes survival, migration, and proliferation of endothelial cells and induces fenestrations that increase vascular permeability.<sup>17</sup> Moreover, VEGF enhances the proliferation and migration of keratinocytes.<sup>18</sup> Vascular remodeling is regarded as a hallmark of many chronic inflammatory skin diseases, such as psoriasis, systemic lupus erythematosus, systemic sclerosis, dermatomyositis, polyarteritis nodosa, pemphigoid, and pemphigus vulgaris.<sup>19</sup> Most studies have demonstrated statistically

higher VEGF levels in psoriasis and systemic lupus erythematosus than in controls, and levels have correlated with clinical severity.<sup>20,21</sup>

Our observations demonstrate, for the first time, that serum concentrations of VEGF correlate with clinical intensity of AD by total SCORAD index and by objective SCORAD in which subjective symptoms, such as itch and sleeplessness, are excluded. It suggests that VEGF, in some patients, could serve as a useful marker of the severity of AD. It is well known that the IgE-mediated allergic reactions in patients with AD are induced by the mutual interaction of IgE, mast cells, and allergens. Findings indicate that mast cells have an intrinsic capacity to produce and secrete VEGF that can be augmented through the IgE receptor FcεRI. This release of VEGF from activated mast cells enhances vascular permeability and induces vascular endothelial cell proliferation.<sup>22</sup> However, Kochy-Baron *et al.*<sup>14</sup> did not reveal a correlation between VEGF and IgE levels, nor did we. However, in our study, serum levels of total IgE did correlate with total SCORAD, as is generally accepted.<sup>16,23</sup>

Some authors claim that VEGF may influence the pathogenesis of respiratory allergic diseases. Allergic rhinitis and asthma often coexist and affect the clinical course of AD.<sup>24</sup> Our study did not reveal any differences between VEGF levels in patients with AD, with and without other respiratory atopic diseases; however, allergic rhinitis was in an inactive stage in most of our studied patients. It is generally thought that in these patients, VEGF serum levels increase after allergen exposure.<sup>25</sup> Literature data indicate a statistical correlation between the clinical intensity of asthma and VEGF levels in bronchoalveolar lavage but not in patients' serum.<sup>26</sup>

The predisposition to skin infections is included as a minor AD diagnostic criteria according to Hanifin and Rajka.<sup>7</sup> *Staphylococcus aureus* is regarded as the main pathogen, and the density of bacteria colonization correlates with AD skin lesion severity. These antigens bind to the common variable chains of T-cell receptors and provoke a polyclonal activation of T cells. In addition, staphylococcal superantigens induce the production of superantigen-specific IgE. Binding of superantigen-specific IgE with superantigen leads to basophil activation.<sup>27</sup> T lymphocytes and basophils are the source of VEGF, and their activation influences VEGF levels.<sup>5</sup> However, in our study, about 60% of examined patients had a history of recurrent skin infections but during evaluation did not have any clinical symptoms of skin infection, and that may explain the lack of correlation between VEGF level and infection in our study.

Chronic inflammation is characterized by increased T-helper 1 activity and increased levels of IL-8, IL-15,

and TNF $\alpha$ .<sup>28–30</sup> In our study a significant decrease of IL-8 was observed in patients with AD compared to the healthy control. We also found that the mean serum concentration of TNF $\alpha$  was significantly higher in patients' AD than in the control group. *In vitro* TNF $\alpha$  inhibits endothelial cell proliferation, but *in vivo* stimulates neo-vascularization.<sup>31,32</sup> Moreover, literature data demonstrate a dual role of TNF $\alpha$  in angiogenesis, where at low levels it promotes angiogenesis, whereas at high concentrations it inhibits endothelial cell proliferation.<sup>32</sup>

RANTES, a member of the C-C chemokine family, is a potent chemoattractant for eosinophils, monocytes, basophils, and lymphocytes at the site of inflammation with a particular preference for memory T cells and eosinophils.<sup>33,34</sup> Augmented production of RANTES has correlated with inflammation associated with AD.<sup>35</sup> Moreover, findings suggest a role for staphylococcal superantigens in the production of RANTES during the development of atopic skin inflammation.<sup>36</sup>

Some studies have suggested a proangiogenic role of IL-6,<sup>4,37</sup> but in our study, we did not find any correlations between IL-6 levels in AD versus healthy individuals.

We did not find any correlation between AD severity and serum concentrations of IL-6, IL-8, IL-15, TNF $\alpha$ , or CCL5 (RANTES).

Statistically significant higher levels of VEGF in patients with AD and its correlation with disease severity suggest a possible role for this factor in angiogenesis and the development of AD. VEGF may influence vascular remodeling and vascular permeability, and this may increase edema facilitating the recruitment of more inflammatory cells to the area of inflammation.

We note that the commercially available ELISA that we used to measure VEGF-A does not distinguish between VEGF and its anti-angiogenic isoform VEGF<sub>165b</sub>. Thus, the possibility exists that measurement of VEGF also detects some variants with anti-angiogenic activity.<sup>38</sup>

We conclude that VEGF may potentially be an important mediator in the pathogenesis of AD, as VEGF levels correlate somewhat with AD severity, as measured by SCORAD index ( $r = 0.31$ ;  $P < 0.05$ ) and objective SCORAD ( $r = 0.25$ ;  $P < 0.05$ ). A larger number of patients with AD should be similarly analyzed to overcome the limitations of our small number of patients with AD. Downregulation of VEGF production or blockade of VEGF action could possibly be a mechanism to target, as a novel therapeutic strategy for a variety of inflammatory skin diseases, including AD.

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