Peripheral blood eosinophilia in atopic dermatitis

D. Jenerowicz, M. Czarnecka-Operacz, and W. Silny

ABSTRACT

Background. Eosinophil granulocytes and eosinophilic granule proteins are deposited in the skin lesions of AD patients. Increased Th2 activity in the acute phase of AD is associated with IL-5 expression and results in enhanced eosinophilopoiesis, eosinophilic activation, and chemotaxis.

Material and methods. Thirty AD patients attending the Allergic Diseases Diagnostic Center and hospitalized in the Department of Dermatology were examined. Two control groups were included: 30 patients suffering from chronic urticaria and 30 healthy individuals without any symptoms of allergic diseases. Laboratory tests included the number of eosinophils expressed as a percentage of a differential white blood cell count, the absolute number of eosinophils in mm^3 (Carpentier's eosin method), and an evaluation of antigen-specific IgE (asIgE) in serum using fluoroenzyme immunoassay (CAP System FEIA, Pharmacia).

Results. The number of eosinophils/mm^3 in the peripheral blood was 290.0 ± 205.7 in AD patients, and 113.3 ± 93.7 and 153.3 ± 113.7 in the two control groups; the difference was statistically significant. Patients with severe AD had higher eosinophilia than patients with mild to moderate AD, but the difference was not significant. In AD patients with positive SPT tests and detectable specific IgE in serum, and also in patients with symptoms of other atopic diseases, the peripheral blood eosinophilia was more prominent compared to patients with negative SPTs and without symptoms of other atopic diseases.

Conclusion. The results point to the role of eosinophils in etiopathogenesis of AD. Peripheral blood eosinophilia could serve as a diagnostic parameter in differentiating allergic AD from non-allergic AD.

KEY WORDS

peripheral eosinophilia, atopic dermatitis, chronic urticaria

Introduction

AD is a chronic, relapsing skin disease, usually beginning in early childhood. The clinical manifestations and locations of skin lesions depend on age, but the main symptom are intense pruritus, causing emotional distress and sleep disturbances (1–3). AD patients are genetically predisposed to increased synthesis of IgE antibodies specific for airborne and food allergens (4).

Eosinophil participation in etiopathogenesis of AD

is still discussed. Th2 derived IL-5 induces formation, activation, and chemotaxis of eosinophils. The importance of IL-5 for the generation of eosinophils is evident from studies in IL-5–deficient mice, which are unable to develop eosinophilia upon allergic sensitization and challenge (5). IL-3 and GM-CSF have also been shown to induce eosinophil production in the bone marrow. Eosinophils generate leukotrienes (LTC4, LTD4, LTE4) that amplify the inflammatory cascade, probably by acting as chemotactic factors or by triggering the release of cytotoxic proteins (6).

According to some authors (7), eosinophil degranulation takes place directly within skin lesions. On the other hand, Karawajczyk et al. (8) suggest that it could occur in the blood, in the bone marrow, or both. After degranulation, a striking extracellular deposition of substances such as eosinophil-derived neurotoxin (EDN), eosinophil cation protein (ECP), or major basic proteins (MBP) appear in AD lesions. An elevation of granule protein levels in peripheral blood correlating with disease activity can be observed. Moreover, in the IgE-mediated late-phase reaction, extracellular eosinophil granule protein deposition corresponds to electron-microscopic observations revealing disruption of eosinophils and free granules in the tissue (7).

Eosinophils also modulate allergic inflammation (6). They are likely to either augment or maintain the Th2 allergic response, particularly IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-16, GM-CSF, eotaxin, and RANTES. IL-12 promotes a switch from a Th2 to Th1 immune response commonly seen at chronic stages of AD (6). According to some authors, this correlates well with disease severity, while others consider it a rather variable parameter (9). The data on increased peripheral blood eosinophilia in patients suffering from AD and concomitant respiratory symptoms are scarce.

The purpose of this study was to investigate the correlation of peripheral blood eosinophilia in AD patients with the clinical severity of the disease and atopy.

Material and methods

Thirty AD patients from 8 to 60 years old (mean 24.5 years), 22 females and 8 males, diagnosed by the Hanifin and Rajka criteria (10), were included in the study. Fifteen patients expressed additional manifestations: atopic asthma (4 patients), allergic rhinitis (11 patients), and allergic conjunctivitis (12 patients). Based on the clinical evaluation of AD patients, two subgroups were selected: subgroup I: 17 AD patients, W-AZS value < 50 points (mild and moderate AD) and subgroup II: 13 AD patients, W-AZS value ≥ 50 points (severe and very severe AD).

The two control groups included 30 patients suffering from chronic urticaria (group A) and 30 healthy individuals (group B); they matched the AD group in age and gender. Detailed disease histories were obtained, regarding the onset and course of AD, factors exacerbating inflammation, disease activity, and further atopic symptoms, as well as the family history of atopy.

Clinical evaluation of AD patients was based on the W-AZS index as proposed by Silny (11), which grades the severity of pruritus, sleep disturbances, and the extent and severity of skin inflammation. The W-AZS is a relatively objective clinical scoring system for AD patients. It evaluates both objective and subjective criteria during all phases of the disease, describes involvement of skin regions, and allows monitoring of the course of the disease (12). In the chronic urticaria patients, both the skin lesions and severity of pruritus were evaluated, using criteria proposed by Lorette (13) and Thomson (14).

SPTs were performed in the group of patients with AD and in control group B, using the set of airborne allergens by Nexter/Allergopharma (grass pollen, tree pollen, weed pollen, feathers, animal dander, molds, and house dust mites *(Dermatophagoides pteronyssinus* and *D. farinae*). The serum level of antigen-specific IgE (asIgE) was assayed using the fluoroenzyme immunoassay (CAP System FEIA, Pharmacia) in selected AD patients. In control group A, SPTs were performed only if the history suggested the possible involvement of airborne allergens. Histamine hydrochloride (1:1000) was used as a positive control and 0.9% saline solution as a negative control. A positive SPT result (+++) was considered if the wheal corresponded to the mean diameter of the histamine wheal (15).

Laboratory tests included differential white blood cell count with eosinophils expressed as percentages and the absolute eosinophil count in mm^3 of peripheral blood (16).

The study was approved by the Ethics Committee of the University of Medical Sciences in Poznañ (statement number 502/03).

Statistical analysis

For numeric variables, descriptive statistics were reported as mean ± standard deviation. Statistical evaluations were performed using ANOVA analysis with post-hoc Newman-Keuls tests. To assess correlations, Spearman's rank correlation coefficient was calculated.

[1] In order to construct a ROC curve, the sensitivity and specificity of the test must be calculated for each possible cut-point value. The X-axis is 1 minus the specificity and the Y-axis is the sensitivity. An index of the adequacy of the test is the area under the curve; a perfect test has an area of 1.0, and a nondiscriminating test has an area of 0.5.

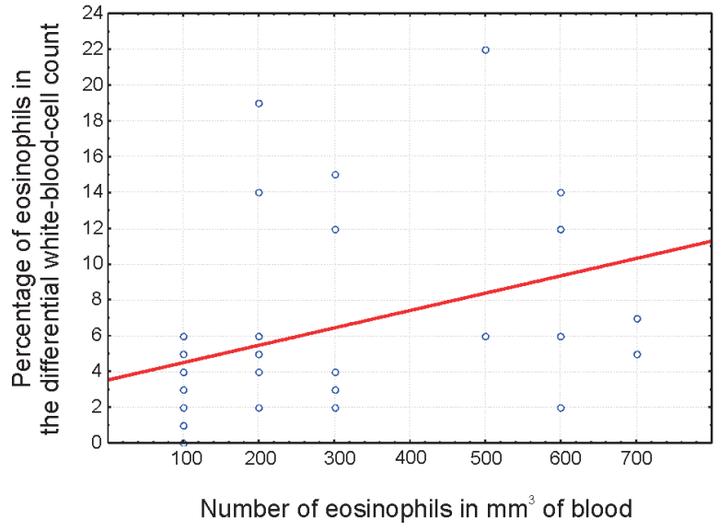

Figure 1: Positive correlation between the number of peripheral blood eosinophils evaluated by eosin method of Carpentier and the percentage of eosinophils in the differential white-blood-cell count in AD patients.

The ability of measured parameters to discriminate between AD patients and healthy individuals was evaluated using ROC (Receiver Operating Characteristic) curves.[1] Statistical analysis was carried out with STATISTICA v. 6.0 and Instat v. 3.0 by GrafPad.

Results

SPTs were positive in 80.8% of AD patients; house dust mites and grass pollen allergens prevailed. Seventy-two percent of the patients were sensitized to multiple allergens (polyvalent allergy) and 28% were sensitized to only one group of allergens (monovalent allergy). In control group B, the SPTs were positive in 26.7%, with the majority sensitized to grass pollen; 25% of these were sensitized to multiple allergens and 75% to only one tested allergen.

The mean absolute eosinophil count was 290.0 ± 205.7 eosinophils/mm³ in AD patients, 113.3 ± 93.7 eosinophils/mm³ in control group A, and 153.3 ± 113.7 eosinophils/mm³ in control group B. Statistical analysis revealed a significant difference with $p < 0.001$ for group A and $p < 0.05$ for group B. The eosinophil percentage in AD patients was 6.3 ± 5.6%, in control group A 2.2 ± 1.9%, and in control group B 3.2 ± 2.8%. The difference between AD patients and control group A was statistically significant ($p < 0.01$), but not with respect to control group B (Table 1).

In the group of patients suffering from AD, the number of eosinophils correlated significantly with the percentage of eosinophils in the differential blood cell count (Figure 1).

There was no statistically significant difference between mean eosinophil numbers in the subgroup with mild and moderate AD (288 ± 196.4), and the subgroup with severe AD with 292.3 ± 225.3 eosinophils/mm³. Similarly, there was no statistically significant difference between the mean eosinophil percentage in the differential white cell count: 5.3 ± 4.4% in subgroup I, and 7.7 ± 6.9% in subgroup II.

The mean absolute eosinophil count in AD patients with symptoms of other atopic diseases was 293.3 ± 205.2 eosinophils/mm³ and exceeded the number of eosinophils in AD patients without symptoms, which was 286.7 ± 213.3; the difference was not statistically significant. The mean eosinophil percentage in AD patients with additional atopic manifestations was higher than in group without further signs of atopy: 7.3 ± 6.4% and 5.3 ± 4.7%, respectively, but this was not statistically significant.

Table 1: Mean level of peripheral blood eosinophilia estimated with the use of absolute eosinophil count (Carpentier's eosin method) and in the differential white-blood-cell count in AD patients and control groups.

	AD patients n=30	Control group A n=30	Control group B n=30	Significance
Mean level of peripheral blood eosinophilia (absolute eosinophil count in mm³) x±SD	290±205,7[a]	113,3±93,7[b]	153,3±113,7[c]	a/b- p<0,001 a/c-p<0,05 b/c- NS
Mean eosinophil percentage in the differential white-blood-cell count (%) x±SD	6,33±5,6[a]	2,23±1,9[b]	3,23±2,8[c]	a/b- p<0,001 a/c- NS b/c- NS

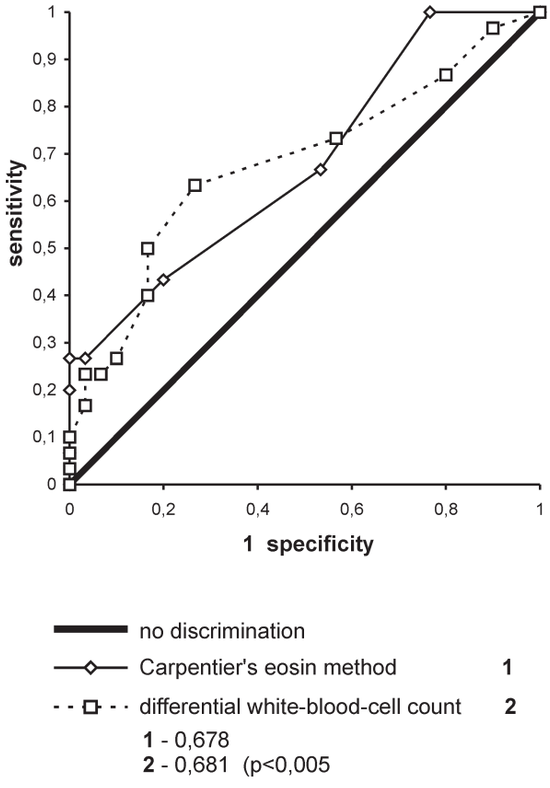

Figure 2: ROC curve analysis of peripheral blood eosinophilia by Carpentier's eosin metod and by differential white-blood-cell count in AD patients and in healthy controls.

In AD patients with positive SPTs and detectable serum IgE, the mean absolute eosinophil count was higher than in patients with a negative SPT, but the difference was not statistically significant: they showed 316.0 ± 213.5 and 160.0 ± 89.4 eosinophils/mm^3, respectively. Nor did the mean eosinophil count differ significantly.

In order to evaluate the predictability of the measured parameters for distinguishing AD patients from healthy persons, an ROC curve analysis was performed. The area under the curve value for peripheral blood eosinophilia determined using the absolute eosinophil count was 0.678, and for the eosinophil percentage in the differential white blood cell count the value obtained was 0.681 (Figure 2). Both achieved values were statistically significantly different from the border value of the non-discriminating test (0.5).

Discussion

According to some authors (17, 18), there is a positive correlation between the number of eosinophils in peripheral blood and disease severity, whereas others have not observed such a relationship (19, 20). Some researchers regard peripheral blood eosinophilia as a more reliable marker of clinical improvement compared to levels of selected eosinophilic proteins (21). According to Toma et al. (22), infants suffering from severe AD reveal impaired growth, developmental delay, a low serum albumin level, and electrolyte disturbances, and have significantly higher number of eosinophils and eosinophilic nuclear lobes, platelets, and total serum IgE level.

A distinct increase of eosinophils in peripheral blood (> 500/mm^3) was seen in only 6 AD patients, whereas an elevated eosinophil percentage in the differential white blood cell count (> 4%) was determined in 14 patients (46.6%). According to Yamamoto et al. (23), the IL-5 gene may play a role in blood eosinophilia associated with AD.

According to Uehara et al. (9), patients with severe AD and a personal history of respiratory symptoms show a significantly higher incidence of blood eosinophilia than patients suffering from mild AD with only skin lesions. On the other hand, Wütrich et al. (24) and Kang et al. (25) found no difference in the blood eosinophil counts between patients with AD and concomitant atopic diseases compared to subjects with pure AD. It is worth emphasizing that, of accompanying atopic manifestations, allergic rhinitis and allergic conjunctivitis prevailed, while asthma was present in only four cases. Our results suggest that in AD patients with other symptoms of atopy, even with allergic conjunctivitis, an increased number of peripheral blood eosinophils may be expected.

There are only a few reports comparing eosinophilia in non-allergic (intrinsic) and allergic (extrinsic) AD. Rho (26) concluded through immunophenotyping that there were more prominent dermal infiltrates with eosinophils as well as eotaxin immunoreactivity in the extrinsic AD. We made a similar observation in the peripheral blood by assessing eosinophilia in patients with both types of AD.

The evaluation that was performed using ROC curves to assess the value of both the total eosinophil count and the percentage of eosinophils in order to distinguish AD patients from healthy persons revealed a statistically significant difference between the area under the curve and the border value of 0.5. The area under the curve was slightly higher if the differential white cell count was applied (0.681), as compared to using the absolute eosinophil count (0.678).

Abbreviations

AD – atopic dermatitis
ECP – eosinophilic cationic protein

EDN/EPX – eosinophilic derived neurotoxin/eosinophil protein X
GM-CSF – granulocyte-macrophage colony stimulating factor
IL- (2-16) – interleukins (2-16)
LTC_4 – leukotriene C_4
LTD_4 – leukotriene D_4
LTE_4 – leukotriene E_4
MBP – major basic protein
RANTES – regulation on activation; normal T-cell expressed and secreted ROC curve
SPT – skin prick tests
Th1, Th2 – T helper 1, T helper 2

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