



Indian J Dermatol. 2019 Jan-Feb; 64(1): 34–40.

PMCID: PMC6340239

doi: 10.4103/ijid.IJD_509_17; 10.4103/ijid.IJD_509_17

PMID: [30745633](#)

Evaluation of Peripheral Blood Eosinophilia in Adolescent and Adult Patients Suffering from Atopic Dermatitis and the Relation to the Occurrence of Allergy to Aeroallergens

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Received 2017 Oct; Accepted 2018 Jul.

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Abstract

Background:

Eosinophils play an important pathogenic role in atopic dermatitis (AD), but its exact function remains to be determined.

Aims and Objectives:

The aim of this study was to correlate the eosinophil count with the manifestations such as asthma bronchiale, rhinitis, level of total IgE, sensitization to mites, animal dander, bird feather, dust, mixture of grass, mixture of trees, mixture of fungi, duration of lesions (persistent or occasional during last year), family history about atopy, and onset of AD (under or above 5 year of age).

Materials and Methods:

Two hundred and seventy-two patients suffering from AD at the age of 14 year or older were examined – 100 men and 172 women with the average age of 26.7 ± 9.5 years and with the average SCORAD index of 32.9 ± 14.1 . Complete dermatological and allergological examinations were performed in all patients with the evaluation of monitored manifestations. Mann–Whitney test for difference in medians was used for statistical evaluation.

Results:

The count of eosinophils in peripheral blood was significantly higher in patients with total IgE ≥ 200 IU/ml, with sensitization to dust, with persistent eczematous lesions and in patients with the onset of AD under 5 year of age. The count of eosinophils above 5% was recorded as well in patients suffering from asthma bronchiale, rhinitis, sensitization to mites, and in patients with positive family history about atopy, but the difference was not significant. On the other hand, the count of eosinophils was under 5% in patients with sensitization to animal dander, bird feather, mixture of grass and trees.

Conclusion:

Our results demonstrated the difference in the count of eosinophils in peripheral blood according to different manifestations in patients suffering from AD.

KEY WORDS: *Animal dander, atopic dermatitis, bird feather, dust, inhalant allergens, mixture of fungi, mixture of grass, mixture of trees, peripheral blood eosinophilia, sensitization to mites*

Introduction

Atopic dermatitis (AD) is a chronic, noncontagious inflammatory skin disease with specific immune and inflammatory mechanisms; it is inflammation of the epidermis and dermis with characteristic clinical and dermatopathological signs.

Eosinophils may play a critical role in the skin lesions of both extrinsic and intrinsic AD patients, and tissue eosinophilia has been shown to be a feature of acute and chronic AD and to correlate with disease activity.[1,2] Eosinophils are cells of the immune system that are most commonly known for their role in defense against parasites and along with basophils and mast cells, as mediators of allergy and asthma. They are derived from CD34+ hematopoietic progenitor cells in the bone marrow called eosinophil/basophil colony-forming unit and represent approximately 1%–6% of white blood cells.[3] In response to the diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci where they modulate immune responses through an array of mechanisms. They can persist in the circulation for 8–12 h and in tissue for 8–12 days in the absence of stimulation.[4,5] Decades ago, eosinophils were thought to have a protective role in the pathogenesis of allergic diseases. Research in the 1980s demonstrated that eosinophils are in fact active pro-inflammatory cells. Granular proteins released by eosinophils play an important role in the pathogenesis of allergic diseases. Under the physiologic conditions, the skin does not harbor eosinophils, but in some of the disease processes, such as AD, these cells can be found in the lesional skin.[6] Eosinophils are also closely associated with the pathogenesis of other atopic diseases, specifically in the respiratory tract, with the development of allergic asthma.[7] Increase in eosinophils in the tissues, blood, and bone marrow is a hallmark of most asthma phenotypes, and in general, elevated numbers correlate with disease severity (although “noneosinophilic/nonneutrophilic” asthma is characteristic of bacterial, viral, and pollutant triggers).[8] It has been well established that interleukin-5 (IL-5) is one of the most important cytokines for generation of eosinophils.[9] Newer studies have demonstrated that distribution of IL-5 genotype is significantly different among AD patients with eosinophilia and those without. Therefore, IL-5 gene may have a role in blood eosinophilia associated with AD.[10] Eosinophils themselves play an important immunoregulatory role by secreting a large number of cytokines and chemokines.[11] Another potentially important role for eosinophils in AD is based on the study that showed Type 2 T-helper (Th2) cytokines can stimulate eosinophils to produce IL-12.[12] Therefore, eosinophils may promote a switch from a Th2-like immune response, in acute lesions, to a Th1-like immune response, in chronic lesions of AD.[13] There is some evidence that eosinophils, in addition to their proinflammatory role, are also involved in tissue repair and fibrotic processes of allergic inflammation. They may play this role by promoting collagen synthesis through the action of their secreted transforming growth factors.[14,15] Eosinophilia (i.e., more than 500 eosinophils per microliter of blood) has been shown to be present in most patients with AD and correlate with the disease activity.[16] However, the diagnostic importance of eosinophilia has been unclear. Since increased blood eosinophils may be due to any other allergic processes and some AD patients have normal blood eosinophil levels, blood eosinophil levels are not commonly used for diagnosis purposes.[17] On the other hand, some authors have suggested that peripheral eosinophil counts are more sensitive in relating the improvement of the skin conditions in AD than serum cationic protein levels.[18] Furthermore, more recent studies suggested that peripheral blood eosinophilia could be used as a diagnostic tool in

differentiating atopic AD from nonatopic AD and that AD patients with other symptoms of atopy have increased blood eosinophilia. Recent studies in both adults and children have indicated the more prominent role of eosinophils in sensitized patients than in those not sensitized.[19,20,21,22,23] There are also studies underlining the relation of eosinophil count, eosinophil-associated cytokines, and disease severity.[24,25] Regarding these results, it seems that eosinophils indicate both severe disease and allergic sensitization. The aim of this study was the evaluation of the correlation between the eosinophil count in the peripheral blood and the followed parameters (such as sensitization to mites, animal dander, dust, bird feather, mixture of grass, mixture of pollen, and mixture of fungi) in patients suffering from AD aged 14 year and older.

Materials and Methods

During 2008–2016, 272 patients suffering from AD at the age of 14 year and older were included in the study. The diagnosis of AD was made with the Hanifin–Rajka criteria.[26] The severity of AD was evaluated according to the SCORAD index.[27] The exclusion criteria were long-term therapy with cyclosporin or systemic corticoids, pregnancy, and breastfeeding. Patients having other systemic diseases were excluded from the study as well. Complete dermatological and allergological examinations were performed in patients included in the study. This study was approved by Ethics Committee of Faculty Hospital Hradec Králové, Charles University of Prague, Czech Republic and signed informed consent was taken from every participant. The aim of this study was to evaluate if there was statistically significant difference in eosinophil count between the patients with positive and negative occurrence of the following parameters such as asthma bronchiale, allergic rhinitis, level of total IgE, sensitization to mites, animal dander, bird feather, dust, mixture of grass, mixture of trees, mixture of fungi, duration of lesions (persistent or occasional in last one year), family history of atopy, and onset of AD (under or above 5 year of age).

Eosinophils were measured in the differential white cell count by means of standard microscopic cytology (with a manual review, May-Grünwald- and Giemsa-Romanowsky-type staining, microscope Olympus BX41-product of Olympus, Hamburg, Germany) and flow cytometry (using Sysmex XE-5000, product of Sysmex, Kobe, Japan).

About 5% of peripheral blood eosinophils and more were considered as a higher count according to this laboratory method performed at the Hematology Department of Faculty Hospital Hradec Králové, Charles University of Prague, Czech Republic.

The diagnosis of asthma bronchiale was made according to the results in spirometry at the allergological outpatient department and with history of wheezing. Asthma was diagnosed when there was at least three separate episodes of wheezing, each at least 3 days in duration during the past year.

The evaluation of allergic rhinitis (seasonal or perennial) was made according to the history of recurrent nasal symptoms/rhinitis (recurrent nasal discharge or blockage with attacks of sneezing and itchy eyes).

The serum level of total IgE was measured by the method of FEIA (Pharmacia CAP system, Uppsala, Sweden). The level of total IgE higher than 200 IU/ml was assessed as positive.

Sensitization to mites, animal dander, dust, bird feather, mixture of grass, mixture of trees, and mixture of fungi was determined according to the specific IgE level and to the skin prick test (SPT) results.

Commercial extracts Alyostal (Stallergens, France) was used for SPT. The serum level of the specific IgE was measured by the method of CAP (system FEIA-Pharmacia Diagnostics, Uppsala, Sweden). The level of specific IgE higher than 0.35 U/ml was assessed as positive.

For the evaluation of the duration of AD, the lesions were evaluated as persistent or occasional according to the dermatological examination during one previous year and according to the patient information.

The onset of AD was elicited according to the patient history (the onset of AD under 5 year of age or later).

Family history of atopy was evaluated according to the history given by the patients (the occurrence of allergy, AD, asthma bronchiale, rhinoconjunctivitis in parents, brothers, sisters, and children).

Statistics We evaluated if there was statistically significant difference in count of eosinophils between the patients with positive and negative occurrence of followed parameters such as asthma bronchiale, allergic rhinitis, level of total IgE, sensitization to mites, animal dander, bird feather, dust, mixture of grass, mixture of trees, mixture of fungi, duration of lesions (persistent or occasional in last one year), family history about atopy, and onset of AD (under or above 5 year of age). Mann–Whitney test and Chi-square test were performed for statistical evaluation.

Results

Patients and tested parameters Two hundred and seventy-two patients suffering from AD, 14 years and older were examined – 100 men and 172 women with the average age of 26.7 years (SD 9.5 years) and with the average SCORAD[27] 32.9 (SD 14.1) points.

The occurrence of monitored parameters in percentages in 272 patients with AD is shown in [Table 1](#). The count of eosinophils in patients suffering from AD and the occurrence of manifestations such as asthma bronchiale, allergic rhinitis, level of total IgE, sensitization to mites, animal dander, bird feather, dust, mixture of grass, mixture of trees, mixture of fungi, duration of lesions (persistent or occasional in last one year), family history about atopy, and onset of AD (under or above 5 years of age) are described below.

Asthma bronchiale One hundred and twenty-four patients suffered from asthma bronchiale, in these patients, the average count of eosinophils was 5.87%; no asthma was recorded in 148 patients; and the count of eosinophils in these patients 4.51%. The difference was not statistically significant, $P=0.2481$.

Rhinitis Two hundred and four patients suffered from rhinitis, in these patients, the average count of eosinophils was 5.42%, no rhinitis was recorded in 68 patients, and count of eosinophils in these patients was 4.24%. The difference was not statistically significant, $P=0.2357$.

Total IgE One hundred ninety-four patients had total serum IgE more than 200 IU/ml and the mean eosinophil count was 5.81% whereas among 78 patients who had normal levels of serum IgE had mean eosinophil count of 3.42%. The difference was significant ($P<0.001$).

Sensitization to mites One hundred and sixty-nine patients suffered from sensitization to mites; in these patients the count of eosinophil was 5.69%; no sensitization to mites was recorded in 103 patients; and the count of eosinophils in these patients was 4.20%. The difference was not statistically significant ($P=0.063$).

Sensitization to animal dander One hundred and thirty-three patients suffered from sensitization to animal dander, in these patients, the count of eosinophils was 4.98%; no sensitization to animal dander was recorded in 139 patients; and the count of eosinophils in these patients was 5.27%. The difference was not statistically significant ($P=0.987$).

Sensitization to dust Seventy-eight patients suffered from sensitization to dust, in these patients, the count of eosinophils was 6.41%; no sensitization to dust was recorded in 194 patients; and the count of eosinophils in these patients was 4.61%. The difference was statistically significant ($P=0.0243$).

Sensitization to bird feather Forty-two patients suffered from sensitization to bird feather, in these patients, the count of eosinophils was 4.88%; no sensitization to bird feather was recorded in 230 patients; and the count of eosinophils in these patients was 5.17%. The difference was not statistically significant ($P=0.7160$).

Sensitization to mixture of grass One hundred and eighty-two patients suffered from sensitization to mixture of grass, in these patients, the count of eosinophils was 4.97%; no sensitization to mixture of grass was recorded in 90 patients; and the count of eosinophils in these patients was 5.46%. The difference was not statistically significant ($P=0.6965$).

Sensitization to mixture of trees One hundred and thirty-five patients suffered from sensitization to mixture of trees, in these patients, the count of eosinophils was 4.97%; no sensitization to mixture of trees was recorded in 137 patients; and the count of eosinophils in these patients was 5.29%. The difference was not statistically significant ($P=0.5825$).

Sensitization to mixture of fungi

Eighty-four patients suffered from sensitization to fungi, in these patients, the count of eosinophils was 5.80%; no sensitization to fungi was recorded in 187 patients; and the count of eosinophils in these patients was 4.85%. The difference was not statistically significant ($P=0.3210$).

Duration of lesions One hundred and seventy patients suffered from persistent lesions, in these patients, the count of eosinophils was 5.79%; no persistent lesions were recorded in 102 patients; and the count of eosinophils in these patients was 4.03%. The difference was statistically significant ($P=0.00678$).

Family history One hundred and fifty patients had positive family history of atopy, in these patients, the count of eosinophils was 6.58%; no family history was recorded in 122 patients; and the count of eosinophils in these patients was 4.34%. The difference was not statistically significant ($P=0.50603$).

Onset of atopic dermatitis Two hundred patients had onset of AD under 5 year of age, in these patients, the count of eosinophils was 5.68%; later onset was recorded in 72 patients; and the count of eosinophils in these patients was 3.59%. The difference was statistically significant ($P=0.0045$).

Discussion

This study demonstrated the difference in the count of eosinophils in peripheral blood according to different manifestations among patients suffering from AD. Despite the progress in understanding the immunology of AD, the pathogenesis still remains unclear. The presence of eosinophils in the inflammatory infiltrate of AD has long been known. Eosinophil numbers as well as eosinophil granule protein levels in the peripheral blood are elevated in most AD patients and appear to correlate with the disease activity.[2] These observations point to a potential role of eosinophils in the pathogenesis of AD. [2]

Since some patients exhibit normal blood eosinophil counts despite active AD and since increased eosinophil numbers may be the consequence of additional allergic disorders, the determination of eosinophil number in blood is not a reliable tool in establishing the diagnosis AD.[2]

We performed this study to evaluate the count of eosinophils in peripheral blood in AD patients 14 year and older and compare this count of eosinophils according to the manifestations such as asthma bronchiale, allergic rhinitis, level of total IgE, sensitization to mites, animal dander, bird feather, dust, mixture of grass, mixture of trees, mixture of fungi, duration of lesions (persistent or occasional in last one year), family history about atopy, and onset of AD (under or above 5 year of age).

In this study, the significant difference in the count of eosinophils was recorded between AD patients with the level of total IgE under and above 200 IU/ml, between patients with and without sensitization to dust, between patients with persistent and occasional eczematic lesions and between patients with the onset of AD under and above 5 year of age. Although no significant difference in the count of eosinophils was recorded in evaluating the other parameters, the higher count of eosinophils (5% and more) was recorded in patients with positive occurrence of asthma bronchiale (5.8%), rhinitis (5.42%), sensitization to mites (5.69%), with positive family history about atopy (6.5%), and sensitization to mixture of fungi (5.8%). The interesting results were obtained in evaluating the eosinophil count and sensitization to mixture of grass, trees, animal dander, and sensitization to bird feather. In patients with the positive findings of the sensitization to these aeroallergens, the count of eosinophil was under 5% and was lower in comparison to patients without sensitization to these allergens. In AD patients without asthma bronchiale and rhinitis, with the level of total IgE under 200 IU/ml, with the onset of AD above 5 year of age, in patients without positive family history about atopy and in patients with occasional eczematic lesions, the count of eosinophils was observed normal, it meant lower than 5%. Our results demonstrated that the manifestations such as the level of total IgE, duration of eczematic lesions, sensitization to dust, and onset of AD played important role in the count of eosinophils in peripheral blood.

Regarding the persistent lesions of AD and the higher count of eosinophils, there was some evidence that eosinophils, in addition to their proinflammatory role, were also involved in tissue repair and fibrotic processes of allergic inflammation. They might play this role by promoting collagen synthesis through the action of their secreted transforming growth factors.[14,15]

Significant correlation in eosinophil count in our study was also found between the patients with the level of IgE under and above 200 IU/ml. The elevated IgE response and eosinophilia observed in patients with AD might reflect increased response of Th2 cytokines with a concomitant decrease in interferon-gamma production. However, the cross-regulation of Th1/Th2 derivation and function in AD patients are incompletely characterized. Yoshizawa *et al.* investigated serum levels of several cytokines (IL-18, IL-12, IL-10, IL-2, and IFN- γ) in patients with AD to assess their possible relationship to the severity of disease. Serum IL-18 level in AD patients was significantly higher than in healthy controls and significantly correlated with eosinophil counts and serum soluble IL-2 receptor (sIL-2R) levels.[28] Akan evaluated the risk factors of disease severity to facilitate better management of children with severe AD.[29] Skin prick tests were performed, and percentage of peripheral blood eosinophils, total serum IgE, and specific IgE were measured. The median age of the 501 patients was 15 months, and 315 were male. The patients with severe AD had sensitization to common allergens, food allergens, and eosinophilia more frequently than those with mild-to-moderate disease. According to their results, eosinophilia might predict severe disease and allergic sensitization. Further large-scale follow-up studies are needed to improve the reliability and relevance of this relation.[29] In the Jerenowicz's study, 30 AD patients were examined.[21] 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 sera, 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, but the difference was not statistically significant.[21] A Japanese study found that the eosinophil levels roughly correlated with the disease severity, but the pattern of eosinophilia was not homogeneous.[17] Very high eosinophil counts were common in severe cases of AD who had a personal or family history of respiratory atopy, while normal or moderately elevated counts were obtained in severe cases of "pure" AD who had neither a personal nor a family history of respiratory atopy. It was suggested that disease severity and personal or family history of respiratory atopy are important factors in determining high blood eosinophil levels in AD.[18] Another Japanese study evaluated the role of eosinophils in AD by correlating levels of serum eosinophil cationic protein (ECP), clinical activity, eosinophil count, and IgE level.[30] A positive correlation was observed between the number of peripheral blood eosinophils and serum ECP level in severe cases. Further, the ECP level significantly decreased with clinical improvement of AD or decreased number of blood hypodense eosinophils in anti-allergic drug-treated patients. There was no correlation between serum ECP and IgE levels. These findings indicated that eosinophils might release their granular contents, including ECP, into the peripheral circulation and/or inflammatory skin lesions and subsequently provoke a clinical exacerbation by stimulating allergic reactions.[30] Similarly, Dhar *et al.* found that both the absolute eosinophil count and the IgE level showed significant covariance with disease severity. Their study showed that clinical activity of the disease as recorded by the SCORAD index could be used as an indicator of the hematological abnormalities as well as to some extent as a prognostic indicator. One-way analysis of variance showed a significant association of the absolute eosinophil count and the IgE level with a family history of AD only when both parents were affected. The eosinophil count and the IgE level also showed a significant association with a history of bronchial asthma in patients with AD but not with allergic rhinitis.[31] In our previous studies, we evaluated the count of eosinophils in peripheral blood in patients suffering from AD according to the occurrence of food allergy and food hypersensitivity reactions (FH reactions) and according to the severity of AD (mild, moderate, and severe form according to the SCORAD index). Although no significant difference in the count of eosinophils between patients suffering from mild, moderate, and severe forms of AD was recorded in this previous study, we could observe that the count of eosinophils was normal (4.22%) in patients suffering from a mild form of AD, but this count was higher in patients suffering from moderate and severe forms (5.68% and 6.73%).[32] In another previous study, we evaluated the relation between the count of eosinophils in peripheral blood and the occurrence of FH reactions in AD patients. The difference in count of eosinophils in patients with and without FH reactions was not statistically significant. When evaluating the occurrence of FH reactions to single food, the count of eosinophils was significantly higher only in patients suffering from reactions to carrot.[33]

According to Ring, clinical improvement due to therapeutic intervention was associated with markedly reduced eosinophilic inflammation. Although this observation made it likely that eosinophil played an important pathogenic role in AD, its exact function remained to be determined.[2] According to previous

studies mentioned above and according to our results, peripheral blood eosinophilia could serve as a diagnostic parameter in differentiating the occurrence of other atopic diseases and manifestations.

Conclusion

Our results demonstrated the role of eosinophils in sensitization to different aeroallergens in AD patients and in the etiopathogenesis of AD.

The count of eosinophils in peripheral blood was significantly higher in patients with total IgE above 200 IU/ml, in patients with sensitization to dust, with persistent eczematous lesions and in patients with the onset of AD under 5 year of age. The count of eosinophils above 5% was recorded as well in patients suffering from asthma bronchiale, allergic rhinitis, sensitization to mites and in patients with positive family history about atopy, but the difference was not statistically significant. On the other hand, the count of eosinophils was under 5% in patients with sensitization to animal dander, bird feather, and mixture of grass and trees.

Peripheral blood eosinophilia could serve as a diagnostic parameter in evaluating the severity of disease (duration of lesions) and differentiating the occurrence of other atopic diseases and parameters.

Financial support and sponsorship Nil.

Conflicts of interest There are no conflicts of interest.

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Figures and Tables

Table 1

The frequency of manifestations among atopic dermatitis patients ($n=272$)

Manifestation	Number of patients (%)
Asthma bronchiale (AB)	124 (46)
Allergic rhinitis (RC)	204 (75)
Total IgE (≥ 200 IU/ml)	194 (71)
Sensitization to mites	169 (62)
Sensitization to animal dander	133 (49)
Sensitization to bird feather	42 (15)
Sensitization to dust	78 (29)
Sensitization to mixture of grass	182 (67)
Sensitization to mixture of trees	135 (49)
Sensitization to mixture of fungi	84 (31)
Duration of lesions (Persistent)	170 (62)
Family history (Positive)	150 (55)
Onset of AD (Under 5 year of age)	200 (73)

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