ORIGINAL ARTICLE / ÖZGÜN MAKALE

Turkish Journal of Immunology - Open Access
www.turkishimmunology.org

Turk J Immunol 2018; 6(3):123–130

The Effect of Viral Infections and Allergic Inflammation in Asthmatic Patients on Immunotherapy

Viral İnfeksiyonların ve Allerjik Yangının İmmünoterapi Uygulanan Astımlı Hastalardaki Etkisi

Luljeta AHMETAJ[1], Bakir MEHİC[2], Refet GOJAK[3], Arber NEZİRİ[4]

Abstract

Introduction: The prevalence of allergic asthma are increasing, and the clinical outcome and risk factors of immunotherapy in the treatment of allergy have not been well established. Especially, the impact of viral infection on cytokines in allergic inflammation has yet to be established. This study aimed to determine serum IL-3, IL-11 and IgE levels and blood eosinophil and neutrophil counts during a one-year follow up in patients with allergic asthma on immunotherapy and those on anti-asthmatic drugs only, in the presence of influenza-like illness.

Materials and Methods: Sixty patients with allergic asthma were included in the prospective and comparative clinical study with randomization into two treatment groups. Each patient in the immunotherapy group was treated with subcutaneous specific immunotherapy. After patient recruitment, the serum IL-3, IL-11 and IgE levels and blood eosinophil and neutrophil counts and the frequency of influenza-like symptoms were recorded during a one-year follow up.

Results: A large percentage of patients in the control group had flu symptoms compared to those in the immunotherapy group. The median serum IL-3 and the IL-11 levels were significantly higher in the immunotherapy group of patients compared to the control group. The median serum IgE level was significantly higher in the immunotherapy group of patients compared to the control group during second quarter of follow-up.

Conclusion: The presence of influenza-like symptoms during allergen specific immunotherapy did not significantly change IL-3, IL-11 levels, neutrophil and eosinophil counts.

Keywords: Allergic asthma, IL-3, IL-11, IgE, eosinophils, neutrophils, immunotherapy

Öz

Giriş: Allerjik astımın görülme oranının artmasına karşılık, allerji tedavisindeki risk faktörleri ve immünoterapinin klinik sonuçları tam olarak bilinmemektedir. Özellikle viral infeksiyonların allerjik inflamasyondaki etkileri rolü açıklanmamıştır. Bu çalışmada, immünoterapi veya sadece anti-astım ilaçları alan ve grip benzeri semptomları olan hastalarda bir yıl boyunca IL-3, IL-11 ve IgE seviyelerindeki değişimler irdelenmiştir.

Gereç ve Yöntemler: Allerjik astımı olan 60 hasta iki ayrı tedavi grubuna ayrılarak prospektif ve karşılaştırmalı olarak irdelendi. İmmünoterapi grubundaki her hastaya subkutan olarak özgün immünoterapi verildi. Hastalar çalışmaya alındıktan sonra serum IL-3, IL-11 ve IgE düzeyleri ile influenza benzeri semptomları kaydedildi.

Bulgular: Grip semptomları kontrol grubundaki hastalarda immünoterapi grubundaki olgulara göre daha fazla oranda saptandı. Ortanca IL-3, IL-11 seviyeleri, immünoterapi alan hastalarda kontrol grubundaki olgular ile karşılaştırıldığında daha yüksek idi. IgE seviyeleri ise, immünoterapi alan olgularda sadece 2. üç aylık dönemde kontrol grubuna göre daha yüksek olarak saptandı.

Sonuç: Grip benzeri semptomları olan hastalarda IL-3, IL-11 seviyeleri ile nötrofil ve eozinofil sayıları anlamlı olarak değişmemektedir.

Anahtar Kelimeler: Allerjik astım, immünoterapi, IL-3, IL-11, nötrofil, eozinofil, IgE

[1]Medical Faculty , Physiology and Immunology, Faculty of Medicine, Bulevardi i Deshmoreve pn, Prishtine, Kosovo

[2]Faculty of Medicine, Klinika za plucne bolesti, Sarajevo, Bosnia and Herzegowina

[3]KCU, Klinika za Infektivne bolesti KCU, Sarajevo, Bosnia and Herzegowina

[4]SHSKUK, Urology, Prishtina, Kosovo

Correspondence:
Arber NEZİRİ, SHSKUK, Urology, Bulevardi i Deshmoreve pn, Prishtine, Kosovo

E-mail: luljetaahmetaj@gmail.com

Received: Oct 24, 2018
Accepted: Dec 10, 2018

https://doi.org/10.25002/tji.2018.965

Introduction

The allergic diseases have been increased last two decades.[1] Specific immunotherapy against allergens (SCIT), is also known as desensitization or hypo-sensitization.[2] This immunotherapy approach was found to reduce the allergic episodes in mono-sensitized young children.[3,4] Immunotherapy was reported to reduce bronchial hyperactivity in patients with bronchial asthma[5] However, the exact mechanism regarding how allergen immunotherapy modifies the allergic disease or impair the progression of asthma remains unknown. An essential advantage of the allergen immunotherapy may be its interference

with the pathophysiologic mechanism (s) responsible for mediator release.[6] Induction of peripheral T cell tolerance to allergens is the primary purpose of the specific allergen immunotherapy (SIT). Once peripheral T cell tolerance is triggered, allergen-specific regulatory T cells (Treg) produce elevated levels of IL-10 and TGF-β which are anti-inflammatory cytokines.[7] A report by Akdis et al.[8] on the mechanism (s) of specific immunotherapy showed that the balance between Th2 and Treg cells is decisive for the development or suppression of allergic inflammation. According to "International Consensus on Allergen Immunotherapy II: Mechanisms, standardization, and pharmacoeconomics", SIT induces the immunotolerance, by activating of T regulatory, B regulatory and particularly type 2 innate lymphoid cells.[4]

Reduced activity of mast cells can be attributable to the lack of IL-3 dependent activation; reduced local production of IgE and decreased the production of histamine-releasing factors.[9] In patients with asthma, an increase of cells expressing IL-3 mRNA has been reported both in mucosal biopsies and in bronchoalveolar lavage cells.[9]

The interaction between atopy and viral infections appears to be a complicated in which the atopic state can influence response to viral infections of the lower airways. Respiratory tract viruses can damage both ciliated and non-ciliated respiratory epithelial cells resulting in necrosis of the airway epithelium, loss of cilia and impairment of mucociliary clearance.[10] When stimulated by IL-1 and TGF-β1, the IL-11 is produced by fibroblasts, epithelial cells and smooth muscle cells of the human airways.[11] IL-11 might be an crucial regulator of inflammatory and remodelling responses in the asthmatic airway.[12,13] Tang et al.[14] reported that targeted expression of IL-11 in mouse airways leads to a T cell inflammatory response with airway remodelling, local accumulation of myofibroblasts, and airways obstruction.

In humans, IgE immunoglobulin is the dominant antibody response against a select group of allergens, that is described as type 1 hypersensitivity reactions.[15] Basophils and mast cells can produce Th2-cytokines, indicating that they may also have essential roles in the production of IgE.[16,17]

After recruitmens of eosinophils from bone marrow, eosinophils participate in the modulation of immune response, inducing the airway hyper-responsiveness and remodelling, the key characteristic features of asthma.[18] Mepolizumab (as an anti-interleukin-5 monoclonal antibody) represents now a strategic anti-eosinophilic treatment, especially in severe eosinophilic asthma.[19]

It is documented that neutrophils are accumulated in severe forms of chronic severe asthma which is associated with chronic airway narrowing. This chemotactic activity in the serum is inhibited by immunotherapy, even in the presence of symptoms.[20]

IL-11 and IL-3 as multilineage expanders in bone marrow,[21] reduced the bronchial hyper-reactivity in asthmatic patients after treatment of them with specific anti-allergic immunotherapy.[21]

This study was aimed to determine serum IL-3, IL-11 and IgE levels, blood eosinophil and neutrophil counts during a one-year follow up in patients with allergic asthma after immunotherapy or anti-asthmatic drugs only, in the presence of influenza symptoms.

Materials and Methods

This study was performed at the University Clinical Hospital in Prishtina, Kosovo, and in cooperation with specialized Allergology Center Ylli, Pristina. In our study, we have included 60 adult patients with allergic asthma (both genders) and randomized into two treatment groups. Thirty patients (f/m=11/19, age 15–53 years) received immunotherapy (immunotherapy group), and another 30 patients were randomized (f/m=17/13, age 17–52 years) in the control group, were treated with standard pharmacotherapy, without immunotherapy. This study was approved by University of Sarajevo Medical Faculty, School of Medicine Ethical Board (Permit No: 741/10). All participants gave written informed consent for inclusion.

The bronchial asthma diagnosis was established based on clinical history of recurrent wheezing, breathlessness, or cough associated with significant bronchial reversibility (FEV1>12% from baseline) after inhalation of 200μg salbutamol when the baseline was <80% predicted, in addition to positive skin tests and increased serum level of total IgE. FEV1 was also measured during exacerbations of asthma symptoms during the study.

The sensitivity to specific allergens determined by skin prick tests (SPTs) (Allergopharma Joachim Ganzer, Germany). Skin wheal diameter ≥3 mm was considered as positive skin prick test reaction.[22] After the recruitment, the serum IL-3, IL-11 and IgE levels and blood eosinophil and neutrophil counts and the frequency of influenza-like illness (cold and flu symptoms) were recorded during a one-year follow up.

The level of IL-3 and IL-11 were determined from the peripheral blood in both groups using a commercially available ELISA test (Ray Biotech Inc) on SAGA Linear reader. Patient's sera were kept in a freezer at-20°C until analysis. Determination of IL-3 and IL-11 was performed every 3, 6, 9 and 12 months, and the frequency of influenza-like symptoms in these patients were recorded.

The IgE level was determined using the ELISA test (Diagnostic Cortez reagents), eosinophil neutrophil counts were determined in blood. May-Grunwald-Giemsa staining method was used for histopathological evaluation and blood cell counts were performed in every 3 months in patients.

Each patient in the immunotherapy group was treated with subcutaneous specific immunotherapy with Novo Helisen Depot (house dust mites), Allergopharma Joachim Ganzer Inc., Germany.

The inclusion criteria of the patients were a clinical diagnosis of allergic asthma (grade intermittent to moderate), and being adult (i.e., >18 year-old). The exclusion criteria included the presence of other acute and chronic diseases of airways, the presence of other allergic diseases (skin allergies, nutritive allergies), or the presence of acute and chronic diseases of the other organ systems.

Subcutaneous immunotherapy was administered using the following depot scheme:

Vial 0 5 TE/ml 0.1, 0.2, 0.4, 0.8 ml (weekly)
Vial 1 50TE/ml 0.1, 0.2, 0.4, 0.8 ml (weekly)
Vial 2 500TE/ml 0.1, 0.2, 0.4, 0.8 ml (weekly)
Vial 3 5000TE/ml 0.1, 0.2, 0.4, 0.6, 0.8 and1.0 ml (weekly)
(with continued maintenance dose of 1.0 ml) monthly.

We started with an induction phase (weekly injections) and continued with the maximally tolerated dose in the maintenance phase (monthly injections).

We excluded patients with contraindications for immunotherapy treatment such as inhalation of β-2 blockers. We also excluded the patients who had pregnancy, hypersensitivity conditions not exclusively depended on IgE mechanisms, immune complex and autoimmune disease, immunodeficiency, unstable asthma.

Statistical analyses

For statistical analyses of the results of our study, we used an SPSS for Windows statistical program (version 19.0, SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel (Version 11. Microsoft Corporation, Redmond, WA, USA). For the analysis of nominal variables, we used Chi square test. Non-parametric tests (Mann-Whitney U test, Wilcoxon Test) were used. For the statistical significance, the 95% confidence interval was used (p value=0.05). The *p*-value of the statistical test signified for either accepting or rejecting the hypothesis.

Results

Figure 1 shows the percentages of patients with influenza-like symptoms during the study.

At 3rd and 6th months of the study, the median serum IL-3 level in immunotherapy group was not significantly different compared to that of control group (p=0.114 and p=0.174, respectively; Table 1). However, during the at 9th and 12th month of the study the median serum IL-3 levels were significantly higher in immunotherapy group of patients compared to the control group of patients (p=0.003 and p=0.0005, respectively) (Table 1).

In this study sample, there was a significant difference in the serum IL-11 levels between immunotherapy and control groups during first 3 months. Patients in the immunotherapy group had significantly lower serum IL-11 levels compared to that of the control group (p=0.0005).

During 6th month the median serum IL-11 level in the immunotherapy group of patients was not found to be significantly different (p=0.824). At the measurements performed at 9th and 12th month of the study the median serum IL-11 level was significantly higher in immunotherapy

Turk J Immunol 2018; 6(3):123–130

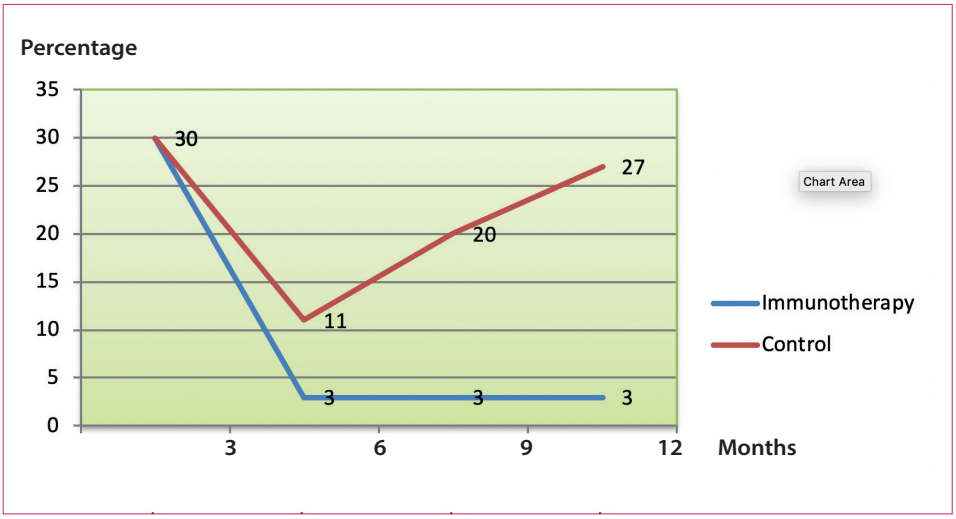

Figure 1. The frequencies of influenza-like symptoms during the one-year follow-up in the control and immunotherapy groups.[21]

Table 1. Serum IL-3 levels at different time points

Time of measurement	Group	N	Minimum	Maximum	Percentiles			Test	*P* value
					25th	50th (Median)	75th	Mann-Whitney U	
3rd month	Immunotherapy	30	4.96	117.80	9.00	12.02	14.77	343.0	0.114
	Control	30	4.10	103.10	9.50	14.25	44.60		
6th month	Immunotherapy	30	9.10	466.00	11.96	14.35	77.15	358.0	0.174
	Control	30	4.50	87.50	11.20	14.61	46.55		
9th month	Immunotherapy	30	11.30	468.70	14.72	46.84	171.8	246.5	0.003
	Control	30	3.50	145.00	11.05	15.50	40.60		
12th month	Immunotherapy	30	11.00	1794.20	20.47	194.25	754.80	172.0	0.0005
	Control	30	3.90	391.00	8.80	13.25	45.90		

group of patients compared that of control group (p=0.003 and p=0.001, respectively) (Table 2).

There was no statistically significant difference in terms of eosinophil counts between immunotherapy and control group during the first six months of the study (p=0.151 and p=0.237, respectively). At the measurements done at 9th month of study the median eosinophil count was significantly higher in the immunotherapy group (p=0.039). At the 12th month of the study there was not a significant difference in terms of eosinophil count between immunotherapy and control groups (p=0.159) (Table 3).

Table 2. Serum IL-11 levels at different time points in immunotherapy and control groups

Time of measurement	Group	N	Minimum	Maximum	Percentiles			Test	*P* value
					25th	50th (Median)	75th	Mann-Whitney U	
3rd month	Immunotherapy	30	0.26	61.20	2.30	3.10	6.80	208.0	<0.0005
	Control	30	0.37	83.60	8.70	12.20	40.80		
6th month	Immunotherapy	30	1.13	82.10	2.97	15.45	47.10	435.0	0.824
	Control	30	2.17	91.24	8.15	11.70	44.26		
9th month	Immunotherapy	30	11.30	468.70	14.72	46.84	171.80	246.5	0.003
	Control	30	3.50	145.00	11.05	15.50	40.60		
12th month	Immunotherapy	30	1.90	1440.40	29.80	176.90	1010.70	217.0	0.001
	Control	30	0.40	310.90	9.42	14.60	60.75		

Table 3. Eosinophil counts in peripheral blood in immunotherapy group of patients at different trimesters and control group

Time of measurement	Group	N	Minimum	Maximum	Percentiles			Test	*P* value
					25th	50th (Median)	75th	Mann-Whitney U	
3rd month	Immunotherapy	30	0.00	0.12	0.02	0.03	0.05	354.0	0.151
	Control	30	0.00	0.09	0.01	0.02	0.04		
6th month	Immunotherapy	30	0.00	0.19	0.02	0.04	0.05	371.0	0.237
	Control	30	0.00	9.70	0.01	0.03	0.04		
9th month	Immunotherapy	30	0.00	0.19	0.02	0.04	0.08	312.0	0.039
	Control	30	0.01	0.08	0.02	0.03	0.04		
12th month	Immunotherapy	30	0.00	0.90	0.01	0.04	0.05	356.0	0.159
	Control	30	0.00	0.08	0.02	0.03	0.04		

Table 4. Serum IgE levels in immunotherapy group of patients at different trimesters and control group

Time of measurement	Group	N	Minimum	Maximum	Percentiles			Test	*P* value
					25th	50th (Median)	75th	Mann-Whitney U	
3rd month	Immunotherapy	30	9.50	463.00	83.82	105.00	193.75	359.5	0.181
	Control	30	1.40	841.70	35.35	75.05	199.50		
6th month	Immunotherapy	30	9.50	750.00	68.50	128.25	270.00	306.0	0.033
	Control	30	1.50	769.00	33.30	66.00	170.00		
9th month	Immunotherapy	30	3.30	917.20	57.10	107.80	281.90	373.0	0.255
	Control	30	1.20	670.00	45.37	83.50	174.00		
12th month	Immunotherapy	30	4.30	865.40	42.75	113.50	288.50	401.0	0.469
	Control	30	2.50	657.00	40.05	87.50	235.00	401.0	0.469

Table 5. Neutrophil count in peripheral blood smears in immunotherapy and control group of patients at different trimesters

Time of measurement	Group	N	Minimum	Maximum	Percentiles			Test	*P* value
					25th	50th (Median)	75th	Mann-Whitney U	
3rd month	Immunotherapy	30	0.40	0.82	0.54	0.64	0.68	12.5	0.283
	Control	30	0.20	0.83	0.54	0.59	0.65		
6th month	Immunotherapy	30	0.40	0.89	0.50	0.55	0.66	386.5	0.345
	Control	30	0.23	64.0	0.55	0.60	0.65		
9th month	Immunotherapy	30	0.40	0.89	0.51	0.56	0.64	287.5	0.016
	Control	30	0.53	157.00	0.55	0.65	0.66		
12th month	Immunotherapy	30	0.36	0.87	0.48	0.54	0.62	196.5	<0.0005
	Control	30	0.45	0.85	0.63	0.65	0.67		

Unlike changed eosinophil counts, serum IgE levels did not differ in patients undergoing immunotherapy at 3rd, 9th and 12th month of the study (p=0.181; p=0.255; p=0.469, respectively). However, the median serum IgE level was, significantly higher in the immunotherapy group measured at the sixth month of study (p=0.033) (Table 4).

There was no significant difference in the neutrophil counts between immunotherapy and control groups at 3rd and 6th month of the study (p=0.283 and p=0.345, respectively). when analyzing the neutrophil counts performed at 9th and 12th month of the study it was significantly lower in immunotherapy group compared to the control group of patients (p=0.016 and p=0.0005, respectively) (Table 5).

Discussion

This prospective and comparative clinical study indicated that allergen-specific immunotherapy had no influence

on standard chemistry and hematology parameters (eosinophils, neutrophils and total IgE) in the presence of influenza-like symptoms.

The prominent source of IL-3 appears to be activated T cells although other cell types have also been reported to produce it.[13] IL-3 play a role in allergic diseases involves the inhibition of basophil apoptosis via PI3K *in vitro* but has little effect on basophil's survival *in vivo.* In this study, the mean serum IL-3 levels during 9th and 12th month in patients treated with immunotherapy were significantly higher then control group (Table 1). There was no information in the present literature if this relationship has any impact on clinical practice.

Stromal cells including fibroblasts, epithelial cells, endothelial cells, vascular smooth muscle cells, synoviocytes, osteoblasts, and several tumour cell lines can produce IL-11. Although each cytokine is sufficient to stimulate IL-11 expression, IL-1α and TGF-ß synergistically augment the production of IL-11.[11] Moreover, several tissue-specific stimuli of IL-11 synthesis has been described. For instance, parathyroid hormone, hepatocyte growth factor, or viral infections are shown to induce the production of IL-11 in osteoblasts and airway smooth muscle cells, respectively. [11] Histamine and eosinophil major essential protein further enhance IL-11 expression, whereas IL-6, IL-4, heparin, and steroids inhibit IL-11 synthesis. [13] During asthmatic inflammation, IL-13 together with respiratory viruses induces the expression of IL-11 in eosinophils and a variety of structural cells in the lung. [11–14]

In this study, serum IL-11 levels were found to be higher in control group at 3rd month of the study, whereas it was higher in immunotherapy group at 9th or 12th months (Table 2). Therefore, the level of IL-11 in sera of patients in the immunotherapy group directly correlates with the reduced frequency of influenza-like symptoms.

According to Wilson et al.,[23] immunotherapy with grass pollen inhibits seasonal increases in eosinophils in the nasal epithelium. It has been reported that patients on immunotherapy have decreased eosinophils in tissues and decreased level of eosinophil releasing mediators.[24] Cappella and Durham[25] have shown that eosinophil cationic protein, instead of eosinophil count, might be useful surrogate of effectiveness of immunotherapy.

In the present study, there was no significant difference in the eosinophil count between immunotherapy and control group throughout the study period. However, eosinıophil count was found to be increased at the 9th month of the study (Table 3). Our results are in concordance with the results from a study by Hafner et al.[26] who analyzed three different studies on different immunotherapies showing. significant differences in terms of 13 hematological parameters measured (including eosinophil count in peripheral blood) and eight chemical parameters between immunotherapy and placebo group of patients.

However, the number of eosinophils and IgE in peripheral blood did not correlate with the decline in the frequency of influenza-like symptoms. Taking into account that allergic asthma is IgE mediated allergy, we aimed to evaluate serum IgE level in patients treated with SCIT and in the control group. The results of our study showed that there was no significant difference in serum IgE level between immunotherapy and control group at 3rd, 9th and 12th months of the study. However, serum IgE levels were higher in the patients receiving immunotherapy.

Eifan et al.[27] have evaluated children with asthma/rhinitis after one-year on SCIT, SLIT or antiasthmatic pharmacotherapy, sensitized to house dust mite. The authors did not find a significant change in IgE levels between or within the group. Yu-Huiet et al.[28] have investigated the effects of SCIT in house dust mite-allergic children with asthma and found a significant decrease in total IgE only after 3 years of extended treatment (from 91.4±29.1 to 77.6±26.4). Des et al., reported in their updated review, that most pediatric SCIT trials found no change in total or serum specific IgE after 3–18 months of treatment.[29] It has been reported that, seasonal increase in specific IgE was attenuated after seven weeks of immunotherapy.[29]

Increased number of neutrophils are found in the airways during the late-phase reaction after an allergen challenge, in some patients who died within hours after an asthma exacerbation in nocturnal asthma, in some patients with long-standing asthma, and in patients with corticosteroid-dependent asthma.[30]

This study did not confirm a significant difference in the neutrophil counts between immunotherapy and control group during the first six months of the study. However, during the last 6 months, the median neutrophil count was significantly lower in immunotherapy group compared to that of control group. The finding correlates with the

frequency of influenza-like symptoms in the control group of patients.

Hafner et al.[26] indicated that allergen-specific immunotherapy had no influence on standard biochemical parameters in clinical studies. In the ALLERSLIT® forte, SLIT 6-grass study, the mean neutrophil percentages in the immunotherapy and placebo group were 60% vs. 58%, respectively. In SCIT rBirch study mean neutrophil count in SCIT group was 55.7% vs. 56.8% in placebo group and in the third pre-seasonal SCIT 6-grass study, neutrophils count in SCIT group was 58.8% while it was 56.6% in the placebo group.

Conclusions

This prospective and comparative clinical study indicated that allergen-specific immunotherapy had no influence on standard biochemical and hematological parameters (eosinophils, neutrophils and total IgE) in the presence of influenza-like symptoms.

The level of IL-11 in sera of patients receiving immunotherapy was negatively correlated with the frequency of influenza-like symptoms. On the other hand, Il-3 levels were found to be positively correlated with influenza-like symptoms.

Abbreviations used
BHR: Bronchial Hyper-Reactivity
IL: Interleukin
IgE: Immunoglobulin E
TGF: Tumor Growth Factor

Acknowledgments: This work was supported in part by a grant to Luljeta Ahmetaj from DAAD.

Ethics Committee Approval: This study was approved by University of Sarajevo Medical Faculty, School of Medicine Ethical Board (Permit No: 741/10).

Informed Consent: All participants gave written informed consent for inclusion.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept: L.A.; Design: L.A.; Supervision: B.M.; Resources: L.A.; Materials: L.A.; Data Collection and/or Processing: L.A.; Analysis and/or interpretation: R.G.; Literature search: A.N.; Writing manuscript: L.A.; Critical Review: A.N.

References

1. Genuneit J, Seibold AM, Apfelbacher CJ, Konstantinou GN, Koplin JJ, La Grutta S, et al. Overview of systematic reviews in allergy epidemiology. Allergy2017;72:849–56. [CrossRef]
2. Calderon MA, Demoly P, Gerth Van Wijk R, Bousquet J, Sheikh A, Frew A, et al. EAACI: A European Declaration on Immunotherapy. Designing the future of allergen specific immunotherapy. Clin Transl Allergy 2012;2:20. [CrossRef]
3. Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, et al. International consensus on allergy immunotherapy. J Allergy Clin Immunol 2015;136:556–68. [CrossRef]
4. Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, et al. International Consensus on Allergen Immunotherapy II. Mechanisms, standardization, and pharmacoeconomics. J Allergy Clin Immunol 2016;137:358–68. [CrossRef]
5. Matsuoka T, Shamji MH, Durham SR. Allergen Immunotherapy and Tolerance. Allergol Int 2013;62:403–13. [CrossRef]
6. Zuberbier T, Bachert C, Bousquet PJ, Passalacqua G, Walter Canonica G, Merk H, et al. GA2LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. Allergy 2010;65:1525–30. [CrossRef]
7. Akdis M, van de Veen W. Allergen Immunotherapy. In: Ratcliffe MJH, editor. Encyclopedia of Immunobiology. Elsevier Ltd. Academic Press; 2016. pp. 313–20.
8. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2007;119:780–9. [CrossRef]
9. Mahajan S, Mehta AA. Role of cytokines in pathophysiology of asthma. Iranian J Pharmacol Therapeut 2006;5:1–14.
10. Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: New rationales for asthma prevention and treatment. Nat Med 2012;18:726–35. [CrossRef]
11. Akdis M, Aab A, Altunbulakli C, Azkur K, Costa RA, Crameri R, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. J Allergy Clin Immunol 2016;138:984–1010. [CrossRef]
12. Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: Stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. J Clin Invest 1996;97:915–24. [CrossRef]
13. Bryder D, Jacobsen SE. Interleukin-3 supports expansion of long-term multilineage repopulating activity after multiple stem cell divisions in vitro. Blood 2000;96(5):1748-55.
14. Tang W, Geba GP, Zheng T, Ray P, Homer RJ, Kuhn C, et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. J Clin Invest 1996;98:2845–53. [CrossRef]
15. Antó JM, Pinart M, Akdis M, Auffray C, Bachert C, Basagaña X, et al. Understanding the complexity of IgE-related phenotypes from childhood to young adulthood: A Mechanisms of the Development of Allergy (MeDALL) Seminar. In: J Allergy Clin Immunol 2012;129:943–954.e4. [CrossRef]
16. Froidure A, Mouthuy J, Durham SR, Chanez P, Sibille Y, Pilette C. Asthma phenotypes and IgE responses. Eur Respir J 2016;47:304–19. [CrossRef]
17. Papathanassiou E, Loukides S, Bakakos P. Severe asthma: anti-IgE or anti-IL-5? Eur Clin Respir J 2016;3:31813. [CrossRef]
18. Liu FT, Goodarzi H, Chen HY. IgE, mast cells, and eosinophils in atopic dermatitis. Clin Rev Allergy Immunol 2011;41:298–310. [CrossRef]
19. Pelaia C, Vatrella A, Busceti MT, Gallelli L, Terracciano R, Savino R, Pelaia G. Severe eosinophilic asthma: From the pathogenic role of interleukin-5 to the therapeutic action of mepolizumab. Drug Des Devel Ther 2017;11:3137–44. [CrossRef]

20. Ciepiela O, Ostafin M, Demkow U. Neutrophils in asthma-A review. Respir Physiol Neurobiol 2015;209:13–6. [CrossRef]

21. Luljeta N-A, Mehic B, Gojak R, Valbona Z, Arber N. The Frequency and Intensity of Bronchial Hyper-Reactivity in Patients with Allergic Asthma on Immunotherapy. Immunome Res 2015;11. [CrossRef]

22. Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG, et al. Practical guide to skin prick tests in allergy to aeroallergens. Allergy 2011;67:18–24. [CrossRef]

23. Durham SR, Walker SM, Varga E-M, Jacobson MR, O'Brien F, Noble W, et al. Long-Term Clinical Efficacy of Grass-Pollen Immunotherapy. N Engl J Med 1999;341:468–75. [CrossRef]

24. Jacobsen EA, LeSuer WE, Willetts L, Zellner KR, Mazzolini K, Antonios N, et al. Eosinophil activities modulate the immune/inflammatory character of allergic respiratory responses in mice. Allergy 2013;69:315–27. [CrossRef]

25. Cappella A, Durham S. Allergen immunotherapy for allergic respiratory diseases. Hum Vaccin Immunother 2012;8:1499–512. [CrossRef]

26. Häfner D, Gödicke V, Narkus A. Allergen specific immunotherapy has no influence on standard chemistry and hematology laboratory parameters in clinical studies. Clin Transl Allergy 2014;4:18. [CrossRef]

27. Eifan AO, Akkoc T, Yildiz A, Keles S, Ozdemir C, Bahceciler NN, Barlan IB. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: An open randomized controlled trial. Clin Exp Allergy 2010;40:922–32. [CrossRef]

28. Hui Y, Li L, Qian J, Guo Y, Zhang X, Zhang X. Efficacy analysis of three-year subcutaneous SQ-standardized specific immunotherapy in house dust mite-allergic children with asthma. Exp Ther Med 2014;7:630–4. [CrossRef]

29. Larenas-Linnemann DES, Pietropaolo-Cienfuegos DR, Calderón MA. Evidence of effect of subcutaneous immunotherapy in children: Complete and updated review from 2006 onward. Ann Allergy Asthma Immunol 2011;107:407–416.e11. [CrossRef]

30. Foley SC, Hamid Q. Images in allergy and immunology: Neutrophils in asthma. J Allergy Clin Immunol 2007;119:1282–6. [CrossRef]