Original article

Role of periostin in evaluating the responsiveness of allergic patients to allergen-specific immunotherapy

Nashwa M. Selim[1], Somia El Sheikh[2], Wafaa S. Metwally[3]

Abstract

***Objectives*:** Allergen-specific immunotherapy (AIT) has been considered the most effective treatment for IgE mediated allergies, especially respiratory allergies. Several biomarkers have been developed to evaluate the clinical efficacy of AIT, yet none of them have been thoroughly validated. So our objective here is to investigate the usefulness of periostin as a biomaker for monitoring the efficacy of allergen immunotherapy.

***Materials and methods*:** This study included 46 healthy non-atopic volunteers and 46 patients with allergic rhinitis (AR). They were sensitized only to date palm pollen. The participants were tested by skin prick test and total serum IgE levels were measured. Serum samples were collected from healthy subjects and allergic patients before and after the one-year AIT. Serum levels of periostin, eotaxin, and sIL-2R were estimated by ELISA. Symptom scores in the allergic patients were also evaluated before and after completing one year AIT.

***Results*:** There is a significant increase in serum levels of IgE, periostin, sIL-2R, and eotaxin in allergic patients as compared to healthy controls**.** Symptom scores, sIL-2R and serum periostin levels were significantly decreased after one-year AIT in AR patients.

***Conclusion*:** Periostin can be used as a biomarker to evaluate AIT efficacy in AR patients.

Keywords: allergic rhinitis; biomarkers; periostin; sIL-2R; allergen specific immunotherapy.

Bangladesh Journal of Medical Science Vol. 21 No. 01 January'22 Page :184-190
DOI: https://doi.org/10.3329/bjms.v21i1.56347

Introduction

Allergen-specific immunotherapy (AIT) is the most effective treatment for immunoglobulin E (IgE) mediated diseases, especially allergic rhinitis (AR), and asthma[1]. AIT is indicated in poorely controlled AR where the specific allergens responsible for the symptoms are known [2]. AIT is associated with reduced symptoms, reduced need for rescue medications, and improved quality of life [3]. Also it can prevent asthma onset in children with AR [4]. It leads to a state of desensitization or tolerance in treated patients via induction of allergen-specific regulatory T cells, switch from IgE to IgG isotypes especially IgG4 which block allergen-IgE interactions, and modulation of several cytokine and chemokine responses[5]. As the current AIT efficacy is judged mainly by the subjective assessment of symptoms[6], biomarkers are urgently needed to add objectivity to the assessment. Furthermore, biomarkers can assist in the development of treatment modalities. These kinds of markers can be cellular (Tregs), humoral (sIgG4 and sIgE), molecular (interleukins), or

1. Nashwa M. Selim
2. Somia El Sheikh
3. Wafaa S. Metwally
   Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt

Correspondence: Wafaa S. Metwally, Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University. Shaibet and Nakareyah, City, State, Postal code, Country: Zagazig, 44519, Egypt.
E-mail: drwafaa.salah@yahoo.com

functional (IgE-FAB) [7]. However, till now there is no reliable biomarker to indicate the clinical efficacy of AIT [5-8].

Periostin is a matricellular protein that is most likely to be produced by fibroblasts[9]. Interestingly, periostin was discovered as a novel mediator in allergic diseases especially allergic airway diseases [10]. It is considered as a promising biomarker to determine the efficacy of biologics of asthma-like omalizumab[11,12]. In this work, we evaluated the role of periostin as abiomarker in allergic patients before and after one year of AIT.

Date palm pollen (*Phoenix dactylifera* , *Pho d 2*) is a major cause of allergy throughout the world. Date palm are commonly planted in the Middle East, neighboring countries (Mediterranean, central Africa, western Asia), Australia, and North America. The most common offending allergens in Egypt are house dust mites and tree pollen[13]. Herein we investigated serum periostin level in AR patients sensitized to date palm pollen only.

Materials and methods

Study design

This study included 46 healthy non-atopic volunteers (ages 21-46 years, 20 females and 26 males) and 46 patients (ages 18–45 years, 19 females and 27 males) with allergic rhinitis (AR) to date palm pollen. Subjects in the non-atopic group were chosen on the basis of these criteria: no history of allergic diseases, and negative skin prick test (SPT). Patients in the allergic group were chosen on the basis of these criteria: history of persistent rhinitis for at least 2 years, positive SPT to date palm pollen (Pho d 2) only (5-mm wheal) and no evidence of treatment with AIT during the previous 10 years. AR patients were enrolled from the Allergy and Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt between September 2018 and September 2019. Flowchart for the study design and follow-up is displayed in figure 1. Exclusion criteria included previous treatment with allergen immunotherapy, the use of immunosuppressive drugs, and the presence of any contraindications to AIT ; infectious disorders, autoimmune diseases, malignancies or other inflammatory disorders.

Diagnosis of allergy

Diagnosis of allergy was verified firstly by a history of exposure to date palm pollen, family history for allergic diseases and careful clinical examination. AR patients recorded the nasal symptoms of rhinorrhea, sneezing, itching and obstruction before and after completing one year AIT. The following scale was used for each symptom scoring: 0 = no symptom, 1 = mild (symptom was of short duration and not annoying), 2 = moderate (symptom was frequently annoying but not interfering with normal daily activity or sleep), or 3 = severe (symptom interfered with normal daily activity or sleep). The total nasal symptom score (TNSS) was the sum of the scores for the individual symptoms. TNSS values (0–12) were categorized as mild (0–4), moderate (5–8), and severe (9–12) [14]. Patients are allowed to take oral second-generation H1-antihistamine as needed for severe allergic rhinitis symptoms. Oral or local corticosteriods were permitted on a restricted basis, after consulting with the study physician, for temporary relief of intolerable symptoms and its use was recorded on the diary card[15].

Skin prick test

After that skin prick test (SPT) was done by utilizing apanel containing house dust mites (Der p 1 and Der f 1), tobacco leaf, ryegrass, cottonwood mix, Aspergillus species mix, Ash mix, and date palm pollen (Pho d 2) (AL, Allergy Laboratories, Inc., USA). SPTs were performed at the volar site of the forearm by the application of one drop of each allergen extract to the skin, at least 3 cm apart. Histamine was used as positive control (AL, Allergy Laboratories) and saline as negative control. The sensitivity of the skin test was estimated by the size of the wheal after 20 min. A wheal diameter 3 mm or greater, accompanied by erythema, was defined as a positive reaction, according to a previously validated protocol[16].

Quantification of serum levels of periostin, eotaxin, and sIL-2R

Serum samples were collected from the healthy control subjects and from AR patients before and after the one-year immunotherapy for quantification of periostin, eotaxin, and sIL-2R by ELISA(Abcam, Cambridge, UK) according to the manufacturer's guidance. Written instructions were given to the patients about the treatment processes. All participants have been informed about the aim and the whole investigation procedures of the study as can be seen in Figure 1, and written consents were taken as well. We were able to recruit about 95% of

the study participants (44 AR patients) after one year immunotherapy. These missed cases were due to noncompliance to the subcutaneous immunotherapy and some had devolped an illness during the study period which interfered with the immunotherapy.

Allergen specific immunotherapy (AIT)

The patients were given subcutaneous injections of standardized date palm pollen (Pho d 2) extracts followed the manufacturers' instructions as follow subcutaneous injections in the posterior portion of the middle third of the upper arm with a 1-mL syringe, were administered twice weekly up to a maintenance dose, afterthat injections were administered twice monthly. The injection period of AIT lasted about 12 months and the maximum tolerated dose of 1:1,000 of 5 % (Pho d 2) pollen extracts preparation was attained in 9 months. Patients were asked to remain under supervision after the injection for a minimum of 30 min to report any symptoms they may have experienced[1].

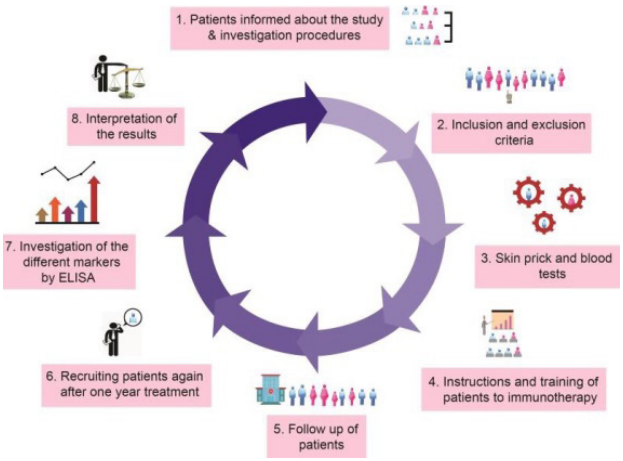

Figure 1: Flowchart for the study design and follow-up during the study period of one-year immunotherapy.

Statistical analysis

Data was analysed by Graph Pad Prism 8 (San Diego, California, USA) and was presented as means and range. Student›s t-test was applied when two values were compared and Chi-square test (x^2) was used to compare two groups regarding the distribution of different variables. Probability values (p) of <0.05 were considered significant. The sample size was calculated by the open epi program with a confidence level of 95 % and power of 80%.

Results

Difference between healthy non-atopic volunteers (n=46) and allergic patients (n=46) as regard serum levels of IgE, periostin, sIL-2R, and eotaxin are shown in table 1. There is significant increase in serum levels of IgE, periostin, sIL-2R, and eotaxin in allergic patients as compared to healthy controls with no significant difference between the two groups as regard age and sex.

Table 1: Difference between healthy non-atopic volunteers and allergic patients as regard serum levels of IgE, periostin, sIL-2R, and eotaxin.

	Healthy non-atopic volunteers	Allergic patients	p
N (female/male)	46 (20/26)	46 (19/27)	
Mean age years (range)	37.7 (21-46)	34.04 (18-45)	0.28
Mean total IgE kU/L (range)	19.5 (6-98)	272 (13-825)	0.001
Mean Periostin concentration ng/ml (range)	31 (14 - 46)	59 (19-125)	0.0001
Mean sIL-2R concentration pg/ml (range)	1860 (1055-2006)	2324 (1155-2977)	0.001
Mean eotaxin concentration pg/ml (range)	109 (60-170)	175 (83-349)	0.0001

The changes in symptom scores after 1 year of AIT in AR patients are presented in Figure 2. All patients were symptomatic with a mean TNSS of 9.2 ± 2.11 at day 0. TNSS scores decreased significantly after one year of AIT (p=0.001) with a mean 2.86 ± 1.42 (Figure 2) and the most common symptom was rhinorrhea (75 %).

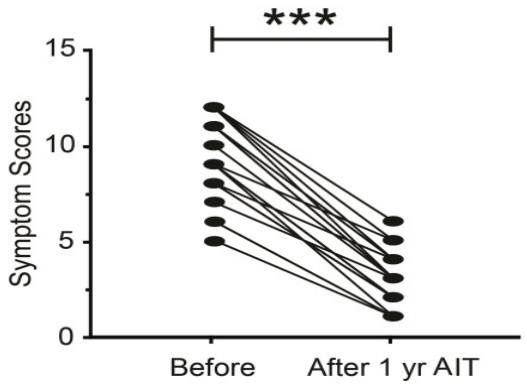

Figure 2: Changes of symptom scores in AR patients after 1 year of AIT.

Serum periostin level of AR patients at the beginning of the study was 59.63 ± 21.53 and decreased significantly (p=0.0001) after one year of SIT with a mean 37.68 ± 12.51 (Figure 3).

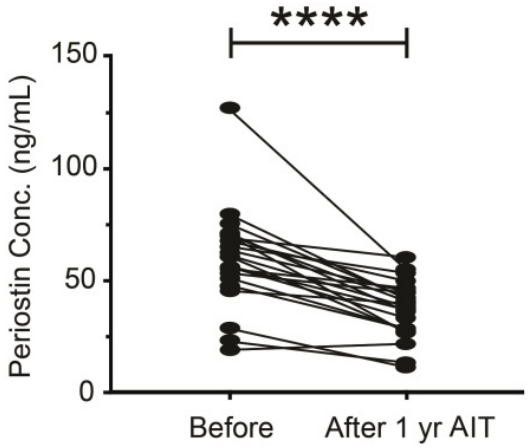

Figure 3: Changes of serum periostin level in AR patients after 1 year of AIT.

Serum eotaxin level has no significant change after one year in AR patients (Figure 4A). As regard sIL-2R, it showed a significant decrease in AR patients with a mean (2324 ± 616.3, 1240 ± 454.9) before and after one year AIT respectively (Figure 4B) (p<0.001).

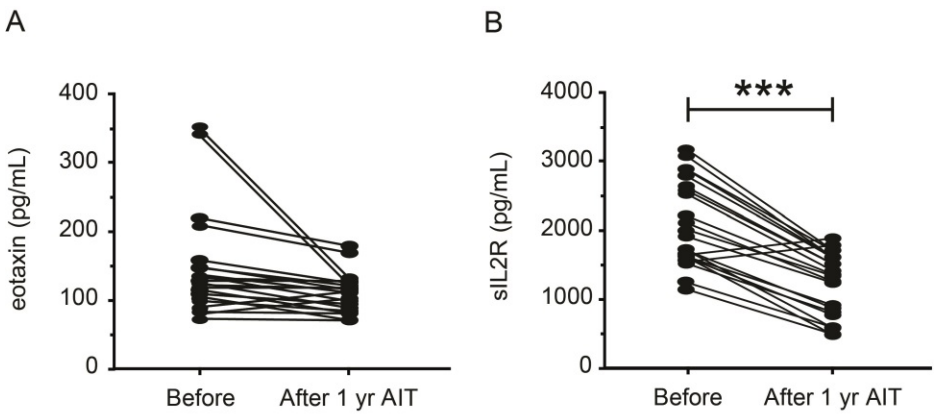

Figure 4: Changes of serum eotaxin and sIL-2R level in AR patients (A, B) after 1 year of AIT, respectively.

Ethical clearance

All procedures were performed in accordance with the Declaration of Helsinki on the treatment of human subjects. This study was approved by Zagazig University Institutional Review Board, Egypt.

Discussion

This study revealed a potential application of the biomarker role of serum level of periostin. According to our results, periostin can be used in evaluating the response of allergic patients to allergen immunotherapy.

The European Academy of Allergy and Clinical Immunology (EAACI) had recognized the need for developing new biomarkers to aid in monitoring the clinical efficacy of AIT and established a task force to review all the candidate biomarkers in trials. However, the resulting report stated that no biomarker indicative of clinical efficacy has so far been identified and validated [3,8].

In our study we found a significant increase in the periostin serum levels of AR patients as compared to healthy non atopic subjects and this results is consistent with the previous studies which has considered periostin as a novel mediator of allergic diseases such as asthma, and AR [9]. Periostin has been utilized to stratify asthmatic patients into Th2-high and Th2-low groups[17]. Interestingly, periostin is a useful marker not only for the diagnosis of asthma but also for monitoring the efficacy of biologics because its level decreased after treatment of asthma patients with omalizumab and rhinitis patients with house dust mite sublingual immunotherapy [11,18]. For AR patients, periostin production was upregulated which resulted in fibrosis and remodelling of the nasal mucosa[10]. Moreover, periostin correlated with the severity and chronicity of AD; and decreased significantly after treatment of AD patients with oral antihistamines and topical corticosteroids [19]. In advanced non–small-cell lung cancer, it was found to be a reliable marker to predict chemotherapy response and survival[20]. Our results in not consistent with those of kim et al. who found that serum periostin levels were not associated with AR in children[21]. This difference may be due to serum periostin levels in non atopic adults were significantly lower than non atopic children so increased basal level of periostin may mask any increase in serum periostin level associated with allergic disease[22] . However, the role of periostin in

monitoring the AIT response hasn't been identified. In the current study, we introduced periostin as a new indicative biomarker for monitoring the clinical efficacy of subcutaneous immunotherapy in AR patients because we found that periostin decreased significantly after one year of AIT in AR patients. During the last few years, the research efforts have recognized some biomarkers that could be helpful in the clinical practice for the diagnosis and prognosis of allergic airway diseases (e.g. IgE, IL-5, IL-6, IL-13, eosinophils, periostin, eotaxin, and sIL-2R). However, the utility of these biomarkers in diagnosis, prognosis and responsiveness to therapy is still controversial[23]. In our study we measured the serum level of eotaxin in both non atopic and AR subjects and found a significant increase in eotaxin serum level in AR patients. Eotaxin is a selective chemokine that has an important role in the pathogenesis of allergic airway diseases and the concordance between an elevated serum level eotaxin and allergen exposure has been implicated in different studies[24]. Our study is consistent with these studies that utilized eotaxin as a plasma marker of allergic inflammation and mucosal eosinophil infiltration [25]. In this study we also found that there is a significant increase in sIL-2R among AR patients as compared to healthy non atopic subjects. Several reports have shown that a soluble form of interleukin 2 receptor (sIL-2R) may result from reactions of immunoregulatory and inflammatory cells also a significant elevation of sIL-2R was observed in atopic dermatitis children [26]. Our results are in agree with these reports in that (sIL-2R) can be utilized as a marker for allergic diseases[27]. Our results showed that there is a significant decrease in symptom scores following AIT treatment in AR patients, which is consistent with other reports[18,28]. In these reports, symptoms scores has decreased significantly after long period treatment with AIT for at least one year.

On one hand, earlier studies for monitoring the immunotherapy efficacy were focused on circulating antibodies, such as specific IgE and IgG antibody level[29,30]. In these studies, variable responses had been observed in total and sIgE with no significant changes during immunotherapy[5,31]. On the other hand, other studies were focused on cell markers and cytokines such as the regulatory cytokine IL10, the inflammatory cytokine eotaxin, and sIL-2R, an indicator of T cells activation[5,32]. However, no clear relationship between serum cytokines and the responsiveness of patients to AIT has been demonstrated[8]. In this regard, we compared some of these cellular biomarkers and cytokines with periostin and found no significant change in eotaxin after one year of therapy in AR patients.These findings were consistent with a previously reported study[33]. We further found a significant decrease in sIL-2R level for AR patients after one year AIT which is consistent with other previous studies [31,32,34].

Conclusion

We can conclude that periostin is an important and novel biomarker for monitoring the clinical efficacy of allergen immunotherapy in AR patients.

Acknowledgement

Authors present their sincere appreciation of all participants and all medical staff of Zagazig University allergy and immunology Unit for their cooperation all through the study

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding Sources

None.

Author Contributions

NMS and WSM : Study design, patient contact, sample collection, laboratory and statistical work, writing manuscript , submission.

SE: Supervision, data analysis, writing and editing manuscript.

References

1. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I , et al. Allergen immunotherapy: a practice parameter third update [published correction appears in J Allergy Clin Immunol. 2011 Mar;127(3):840]. *J Allergy Clin Immunol* 2011;**127**(1 Suppl):S1-S55. http://dx.doi.org/10.1016/j.jaci.2010.09.034

2. Dayasiri K, Thadchanamoorthy V and Thisanayagam U. Diagnosis and Management of Allergic Rhinitis in Children. ***IJHHS*** 2020; **5**(2): 159-162. http://ijhhsfimaweb.info/index.php/IJHHS/article/view/253

3. Compalati E, Braido F and Canonica GW. An update on allergen immunotherapy and asthma. *Curr Opin Pulm Med* 2014;**20**:109–17. http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00063198-201401000-00016

4. Tosca MA, Olcese R, Licari A and Ciprandi G. Allergen immunotherapy and asthma. *Pediatr Allergy Immunol* 2020;**31**(Suppl 24):46–8. https ://doi.org/10.1111/pai.13161

5. Stylianou E, Ueland T, Borchsenius F, Michelsen AE, Øvstebø R, Eirik Mollnes T , et al. Specific allergen immunotherapy: effect on IgE, IgG4 and chemokines in patients with allergic rhinitis. *Scand J Clin Lab Invest* 2016;**76**(2):118–127. http://www.tandfonline.com/doi/full/10.3109/00365513.2015.1110856

6. Pipet A, Botturi K, Pinot D, Vervloet D and Magnan A. Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy. *Respir Med* 2009;**103**(6):800–12. https://linkinghub.elsevier.com/retrieve/pii/S0954611109000146

7. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy*. 2014;**69**(7):854–67. http://doi.wiley.com/10.1111/all.12383

8. Shamji MH, Kappen JH, Akdis M, Jensen-Jarolim E, Knol EF, Kleine-Tebbe J, et al. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI Position Paper. *Allergy* 2017;**72**(8):1156–73. http://www.ncbi.nlm.nih.gov/pubmed/28152201

9. Izuhara K, Nunomura S, Nanri Y, Ono J, Takai M and Kawaguchi A. Periostin: An emerging biomarker for allergic diseases. *Allergy* 2019;1-13. https://onlinelibrary.wiley.com/doi/abs/10.1111/all.13814

10. Ishida A, Ohta N, Suzuki Y, Kakehata S, Okubo K, Ikeda H, et al. Expression of Pendrin Periostin in Allergic Rhinitis Chronic Rhinosinusitis. Allergol Int 2012;**61**(4):589–95. http://linkinghub.elsevier.com/retrieve/pii/S1323893015302604

11. Caminati M, Gatti D, Dama A, Lorenzetti L and Senna G. Serum periostin during omalizumab therapy in asthma: a tool for patient selection and treatment evaluation. *Ann Allergy, Asthma Immunol* 2017;**119**(5):460–2. http://dx.doi.org/10.1016/j.anai.2017.08.004

12. Novosad J, Krčmová I, Bartoš V, Drahošová M, Vaník, P, Růžičková-Kirchnerová O, et al. Serum periostin levels in asthma patients in relation to omalizumab therapy and presence of chronic rhinosinusitis with nasal polyps. *Postepy Dermatol Alergol* 2020; **37**(2): 240–9. https://doi.org/10.5114/ada.2020.94842

13. Postigo I, Guisantes JA, Negro JM, Rodriguez-Pacheco R, DavidGarcia D and Martinez J. Identification of 2 new allergens of Phoenix dactylifera using an immunoproteomics approach. *J Invest Allergol Clin Immunol. 2009*;**19**(6):504–7. http://www.jiaci.org/issues/vol24issue3/5.pdf

14. Schatz M, Meltzer EO, Nathan R, Derebery MJ, Mintz M, Stanford RH, et al. Psychometric validation of the Rhinitis Control Assessment Test: a brief patient-completed instrument for evaluating rhinitis symptom control. *Ann Allergy Asthma Immunol* 2010;**104**(2):118–24. DOI: 10.1016/j.anai.2009.11.063

15. Canonica GW, Baena-Cagnani CE, Bousquet J, Bousquet PJ, Lockey RF, Malling H-J, et al. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. *Allergy* 2007;**62**(3):317–24. http://doi.wiley.com/10.1111/j.1398-9995.2006.01312.x

16. Krouse JH and Mabry RL. Skin Testing for Inhalant Allergy 2003: Current Strategies. *Otolaryngol Neck Surg* 2003;**129**(4_suppl):S33–49. http://journals.sagepub.com/doi/10.1016/S0194-5998%2803%2901398-6

17. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper Type 2–driven Inflammation Defines Major Subphenotypes of Asthma. *Am J Respir Crit Care Med* 2009;**180**(5):388–95. http://www.atsjournals.org/doi/abs/10.1164/rccm.200903-0392OC

18. Hoshino M, Akitsu K, Kubota K and Ohtawa J. *ERJ* 2020; **56** (suppl 64): 236. **DOI:** 10.1183/13993003.congress-2020.236

19. Kou K, Okawa T, Yamaguchi Y, Ono J, Inoue Y, Kohno

M, et al. Periostin levels correlate with disease severity and chronicity in patients with atopic dermatitis. *Br J Dermatol* 2014;**171**(2):283–91. DOI: 10.1111/bjd.12943

20. Zhang Y, Yuan D, Yao Y, Sun W, Shi Y and Su X. Predictive and prognostic value of serum periostin in advanced non–small cell lung cancer patients receiving chemotherapy. *Tumor Biol* 2017;**39**(5):101042831769836. http://journals.sagepub.com/doi/10.1177/1010428317698367

21. Kim DY, Kim JH, Lee KH, Hong SC, Lee HS and Kang JW. Serum periostin level is not associated with allergic rhinitis or allergic sensitization in Korean children. *Int J Pediatr Otorhinolaryngol.* 2017; 93: 24-9. https://doi.org/10.1016/j.alit.2017.11.006

22. Inoue Y, Izuhara K, Ohta S, Ono J and Shimojo N. No increase in the serum periostin level is detected in elementary school-age children with allergic diseases. *Allergol Int*. 2015; 64(3): 289-290. http://dx.doi.org/10.1016/j.alit.2015.04.001

23. Eguiluz-Gracia I, Tay TR, Hew M, Escribese MM, Barber D, O'Hehir RE, et al. Recent developments and highlights in biomarkers in allergic diseases and asthma. *Allergy.* 2018; 73: 2290-2305. http://doi.wiley.com/10.1111/all.13628

24. Ahmadi Z, Hassanshahi G, Khorramdelazad H, Zainodini N and Koochakzadeh L. An Overlook to the Characteristics and Roles Played by Eotaxin Network in the Pathophysiology of Food Allergies: Allergic Asthma and Atopic Dermatitis. *Inflammation*. 2016; 39(3): 1253-1267. DOI: 10.1007/s10753-016-0303-9

25. Yamada T, Miyabe Y, Ueki S, Fujieda S, Tokunaga T, Sakashita M, et al. Eotaxin-3 as a Plasma Biomarker for Mucosal Eosinophil Infiltration in Chronic Rhinosinusitis. *Front Immunol* 2019; **10**:74. doi: 10.3389/fimmu.2019.00074

26. Knipping K, Knippels LMJ, Dupont C and Garssen J. Serum biomarkers for allergy in children. *Pediatr Allergy Immunol* 2017: **28**: 114– 123. https://doi.org/10.1111/pai.12649

27. Ohashi Y, Nakai Y, Tanaka A, Kakinoki Y, Ohno Y, Masamoto T, et al. Serum Levels of Specific IgE, Soluble Interleukin-2 Receptor, and Soluble Intercellular Adhesion Molecule-1 in Seasonal Allergic Rhinitis. *Ann Allergy, Asthma Immunol* 1997;**79**(3):213–20. https://linkinghub.elsevier.com/retrieve/pii/S1081120610630049

28. Karakoc-Aydiner E, Eifan AO, Baris S, Gunay E, Akturk E, Akkoc T, et al. Long-Term Effect of Sublingual and Subcutaneous Immunotherapy in Dust Mite-Allergic Children With Asthma/Rhinitis: A 3-Year Prospective Randomized Controlled Trial. *J Investig Allergol Clin Immunol* 2015;**25**(5):334–42. http://www.ncbi.nlm.nih.gov/pubmed/26727762

29. Di Lorenzo G, Mansueto P, Pacor ML, Rizzo M, Castello F, Martinelli N, et al. Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. *J Allergy Clin Immunol* 2009;**123**(5):1103-10. https://linkinghub.elsevier.com/retrieve/pii/S0091674909003364

30. Gómez E, Fernández TD, Doña I, Rondon C, Campo P, Gomez F, et al. Initial immunological changes as predictors for house dust mite immunotherapy response. *Clin Exp Allergy* 2015;**45**(10):1542–53. http://doi.wiley.com/10.1111/cea.12578

31. Park H-S, Nahm D-H, Kim H-Y, Suh Y-J, Cho J-W, Kim S-S, et al. Clinical and immunologic changes after allergen immunotherapy with Hop Japanese pollen. *Ann Allergy, Asthma Immunol* 2001;**86**(4):444–8. http://dx.doi.org/10.1016/S1081-1206(10)62493-3

32. Ohashi, Nakai, Tanaka, Kakinoki, Washio, Kato, et al. Ten-Year Follow-Up Study of Allergen-Specific Immunoglobulin E and Immunoglobulin G4, Soluble Interleukin-2 Receptor, Interleukin-4, Soluble Intercellular Adhesion Molecule-1 and Soluble Vascular Cell Adhesion Molecule-1 in Serum of Patients on Immunotherapy. *Scand J Immunol* 1998;**47**(2):167–78. https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-3083.1998.00278.x

33. Jahnz-Rozyk K, Targowski T, Glodzinska-Wyszogrodzka E and Plusa T. Cc-chemokine eotaxin as a marker of efficacy of specific immunotherapy in patients with intermittent IgE-mediated allergic rhinoconjunctivitis. *Allergy* 2003;**58**(7):595–601. http://doi.wiley.com/10.1034/j.1398-9995.2003.00083.x

34. Silny W and Czarnecka-Operacz M. Specific immunotherapy in the treatment of patients with atopic dermatitis--results of double blind placebo controlled study. *Pol Merkur Lekarski* 2006;**21**(126):558–65. http://www.ncbi.nlm.nih.gov/pubmed/17405298