



Disponible en ligne sur

ScienceDirect
www.sciencedirect.com

Elsevier Masson France

EM|consulte
www.em-consulte.com



Original article

Changes of basophil activation before and after treatment in children with allergic rhinitis and its clinical significance

Modifications de l'activation des basophiles avant et après traitement chez les enfants atteints de rhinite allergique et sa signification clinique

S. Ma, Y. Qiao*

Department of Otorhinolaryngology, Shanghai Children's Medical Center Affiliated to the Medical School of Shanghai Jiaotong University, 200127 Shanghai, China

ARTICLE INFO

Article history:

Received 16 September 2020

Accepted 10 February 2021

Available online xxx

Keywords:

Allergic rhinitis

Basophil activation test

ABSTRACT

Objective. – To investigate the changes of basophil activation before and after treatment of children with allergic rhinitis and its clinical significance.

Methods. – A total of 18 children with moderate to severe persistent allergic rhinitis in the acute phase were included as the subjects of this study. Based on the conventional therapy for allergic rhinitis, 2 mL of anticoagulated peripheral blood was taken before the treatment (acute phase) and after the remission of clinical symptoms and signs (remission period), respectively. Inhalant allergen stimulation was performed in vitro and basophil activation status (expressions of CD63 and CD203c) was detected by flow cytometry so as to analyze the changes of basophil activation before and after treatment.

Results. – 1. The mean value of VAS score in children with allergic rhinitis before treatment was 8 (6, 9), while that in the post-treatment remission period was 2 (0, 3.2). The symptoms were greatly improved after treatment and the VAS score was decreased significantly. The differences were statistically significant ($P < 0.0001$). 2. The mean expression rate of basophil CD63 (% CCR3+ CD63+) and CD203c (% CCR3+ CD203c+) was 74.35% (52.0%, 81.8%) and 69.2% (43.7%, 81.3%), respectively in pre-treatment acute phase, while that of CD63 (% CCR3+ CD63+) and CD203c (% CCR3+ CD203c+) was 41.5% (24.5%, 80.4%) and 42.1% (15.2%, 81.0%), respectively in post-treatment remission period. The differences were statistically significant ($P < 0.05$).

Conclusion. – According to the published basic and clinical researches, basophils may be important immune cells involved in allergic rhinitis. Basophil activation test can be used as an effective tool for allergen detection. The dynamic observation of basophil activation can provide an immunological basis for the clinical control of allergic rhinitis.

© 2021 Published by Elsevier Masson SAS.

R É S U M É

Objective. – Étudier les changements de l'activation des basophiles avant et après le traitement des enfants atteints de rhinite allergique et sa signification clinique.

Méthodes. – Au total, 18 enfants atteints de rhinite allergique persistante modérée à sévère en phase aiguë ont été inclus comme sujets de cette étude. Sur la base du traitement conventionnel de la rhinite allergique, 2 mL de sang périphérique anticoagulé ont été prélevés avant le traitement (phase aiguë) et après la rémission des symptômes et signes cliniques (période de rémission), respectivement. Une stimulation allergène inhalante a été réalisée in vitro et le statut d'activation des basophiles (expressions de CD63 et CD203c) a été détecté par cytométrie de flux afin d'analyser les changements de l'activation des basophiles avant et après le traitement.

Mots clés :

Rhinite allergique

Test d'activation des basophiles

* Corresponding author.

E-mail address: hwen9308588@sina.com (Y. Qiao).

<https://doi.org/10.1016/j.reval.2021.02.009>

1877-0320/© 2021 Published by Elsevier Masson SAS.

Résultats. – 1. La valeur moyenne du score VAS chez les enfants atteints de rhinite allergique avant le traitement était de 8 (6, 9), tandis que celle de la période de rémission après le traitement était de 2 (0, 3, 2). Les symptômes ont été grandement améliorés après le traitement et le score VAS a diminué de manière significative. Les différences étaient statistiquement significatives ($p < 0,0001$). 2. Le taux d'expression moyen des basophiles CD63 (% CCR3+ CD63+) et CD203c (% CCR3+ CD203c+) était de 74,35 % (52,0 %, 81,8 %) et 69,2 % (43,7 %, 81,3 %), respectivement dans la phase aiguë avant le traitement, tandis que celle de CD63 (% CCR3+ CD63+) et CD203c (% CCR3+ CD203c+) était de 41,5 % (24,5 %, 80,4 %) et 42,1 % (15,2 %, 81,0 %), respectivement dans la période de rémission après le traitement. Les différences étaient statistiquement significatives ($p < 0,05$).

Conclusion. – Sur la base des recherches fondamentales et cliniques publiées, les basophiles peuvent être des cellules immunitaires importantes impliquées dans la rhinite allergique. Le test d'activation des basophiles peut être utilisé comme un outil efficace pour la détection des allergènes. L'observation dynamique de l'activation des basophiles peut fournir une base immunologique pour le contrôle clinique de la rhinite allergique.

© 2021 Publié par Elsevier Masson SAS.

1. Introduction

The incidence of allergic rhinitis (AR) is increasing globally and the incidence of self-reported allergic rhinitis ranges from 2–25% [1,2]. The traditional diagnosis of allergic rhinitis is mainly combined with medical history and allergen examination, such as skin prick test and specific IgE test [3]. The relationship between allergen exposure and symptoms is the necessary condition not only for the diagnosis of allergic rhinitis, but also for the initiation of specific immunotherapy [4].

Currently, symptoms, allergic history and specific IgE test are still main diagnostic method. Most AR patients do not need to detect allergens, but can be relieved by empirical symptomatic treatment [3,5]. According to WHO guidelines, allergens are often detected in AR patients whose symptoms cannot be controlled by oral antihistamines and moderate doses of nasal hormone [6]. Among diagnostic methods for allergic rhinitis, skin prick test and serum IgE test are the main examinations for allergy at present, but both of them have certain limitations [7,8]. IgE-mediated allergic inflammation is the immunological basis of allergic rhinitis [9]. Basophils are important immune cells for IgE-mediated allergic reactions [10]. After allergen/IgE stimulation, basophils activate degranulation and the expression of surface antigens CD63 and CD203C will be elevated [11]. The basophil activation test (BAT) is a method for analyzing the activation degree of basophils after allergen stimulation based on flow cytometry [12]. As reported, the expression levels of CD63 and CD203c in basophils after allergen stimulation could be detected by flow cytometry [13]. However, role of basophil activation in severe allergic rhinitis is still inadequate.

In this study, BAT was used to monitor the activation status of basophils in 18 children with clinically diagnosed allergic rhinitis before and after treatment. The clinical value of BAT in evaluating the therapeutic effect on allergic rhinitis was discussed.

2. Materials and methods

2.1. Case data

The subjects were patients with moderate to severe allergic rhinitis who were clinically diagnosed in the Department of Otorhinolaryngology, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine from December 2017 to December 2018. According to the Chinese society of allergy guidelines for diagnosis and treatment of allergic rhinitis for allergic rhinitis [5], patients had 2 or more symptoms of repeated sneezing, watery discharge, nasal congestion and nasal itching. Pale edema

could be observed in nasal examination and the patients were tested positive for at least one serum inhaled allergen-specific IgE. There were 11 males and 7 females aged 3–13 years old. Among them, there were 3 allergic rhinitis cases combined with asthma and 10 allergic rhinitis cases with a history of atopic dermatitis. All patients included in this study were positive for inhalant allergens. And skin prick test was conducted for all patients. The study was approved by the Ethics Committee of Shanghai Children's Medical Center affiliated to Shanghai Jiaotong University School of Medicine and the informed consent was obtained from all patient's guardians.

2.2. Treatment and follow-up

All patients received routine treatment for allergic rhinitis according to the Chinese Society of Allergy Guidelines including environmental control, pharmacotherapy (like antihistamines and leukotriene receptor antagonists), allergen specific immunotherapy and patient education. All patients were followed up every 2 weeks for 6 months. The symptoms of rhinitis were evaluated and visual analogue scale (VAS) was used in patients at study entry and after remission of the clinical symptoms. Besides, 2 mL of blood was collected in anticoagulant tube from the patients for BAT. At study entry, 2 mL of serum was taken for total IgE and inhalant (house dust, house dust mite, pollen, cat fur, dog fur, mulberry, cockroach, *Ambrosia*) and food-specific (green shellfish, beef, mutton, egg white, milk, shrimp, crab, fish, mango, grass powder, mold, cashew nuts, amaranth) IgE tests (ImmunoCAP). The detection was performed by using an Allergy Screen allergen detector and the matching allergen diagnosis and detection kits (Mediawiss, Germany) strictly according to the manufacturer's instruction. The disease remission was defined according to the Chinese society of allergy guidelines for diagnosis and treatment of allergic rhinitis using the scores of symptoms and scores of clinical characteristics (Tables 1 and 2). The treatment effective index was defined by formula: $[(\text{scores of symptoms} + \text{scores of clinical characteristics before treatment}) - (\text{scores of symptoms} + \text{scores of clinical characteristics after treatment})] / (\text{scores of symptoms} + \text{scores of clinical characteristics before treatment}) \times 100\%$. When the treatment effective index was within 26–66%, it was considered as effective and the disease was considered as remission.

2.3. BAT

The blood sample was stored at 4 °C immediately after collection in anticoagulant tube and underwent BAT within 8 h. The BAT was performed using the BÜHLMANN Flow CAST Basophil Activation

Table 1
The scores of symptoms.

Scores	Nasal itching	Sneeze	Shed tears	Nasal congestion
0	No	No	No	No
1	Occasionally	3~5/once	Blow nose $\leq 4/d$	When inhaling consciously
2	Ant sense but able to bear	6~10/once	Blow nose 5~9/d	Intermittent
3	Ant sense and not able to bear	$\geq 11/once$	Blow nose $\geq 10/d$	Almost all day

Table 2
The scores of clinical characteristics.

Scores	Nasal examination	Nasal mucosa	Nasal secretions
0	Normal	Normal	Normal
1	The inferior turbinate is slightly swollen, and the nasal septum and middle turbinate are visible	Mild pale edema	A little
2	The inferior turbinate is close to the nasal septum or nasal base, and there is a small gap between the inferior turbinate and the nasal base or nasal septum	Pale edema	Moderate
3	The inferior turbinate is close to the nasal septum or nasal base, and no middle turbinate or polyps are found in the middle turbinate mucosa	Obvious pale edema or purplish edema	Filling the nasal cavity

Test kit according to the instructions on the kit. A total of 100 μL of blood with heparin was distributed into three 5 mL flow tubes (positive control, negative control and allergen stimulation sample) and fluorescent antibodies of anti-CCR3 (FITC), anti-CD63 (FITC) and anti-CD203c (FITC) were added into each tube. At the same time, the positive sample was added with anti-Fc ϵ RI Ab (10 μL) stimulation, the negative control was added with PBS as the stimulation control, while the allergen stimulation was added with the inhalant allergen mixture (Inhalant Mix, BÜHLMANN). After incubation at 37 °C for 25 min, the red blood cells were lysed by Red Blood Cell Lysis Buffer (Biyuntian Biotechnology Research Institute Co., Ltd.) for 5 min and washed by PBS. The activation status (SSC lowCCR3^+ CD63+ SSC lowCCR3^+ CD203c+ cell ratio) of basophils were examined using Flow cytometry in a FSCAN flow cytometer (BD Biosciences, USA). Flow data analysis was performed using Cell Quest software under the instructions of BÜHLMANN Flow CAST Basophil Activation test kit. The SSC lowCCR3^+ cell population was identified as basophils in flow cytometry plots in which CD63+ or CD203c+ cells were analyzed as activated basophils.

3. Statistical analyses

All statistical analyses were performed using the SPSS V23.0 software. All data including VAS scores, ratios of cells with positive CCR3 $^+$ CD63 $^+$ (% CCR3 $^+$ CD63 $^+$) and % CCR3 $^+$ CD203c $^+$ were expressed as median (range). Comparisons of VAS scores, % CCR3+ CD63+ and % CCR3+ CD203c+ between the acute phase and the remission phase were performed using a two-sample Wilcoxon rank sum test. $P < 0.05$ was considered statistically significant.

4. Results

4.1. Analysis of serum inhalant allergens

Eleven cases were positive for dust mite allergen, 8 for mixed mold allergens, 3 for mixed tree pollen allergens, 3 for dog dander allergens, 2 for cat dander allergens, 12 for food allergens and 10 cases for 2 kinds of inhalant allergens.

The mean VAS score for children with allergic rhinitis before treatment was 8 (6, 9), and was 2 (0, 3.2) at 6 months after treatment. The symptoms were significantly improved after treatment and the VAS score was significantly decreased ($P < 0.0001$, Table 3 and Fig. 1A).

The median value of CD63 expression rate (% CCR3+ CD63+) and CD203c expression rate (% CCR3+ CD203c+) in basophils stimulated

by allergens at the acute phase was 74.4% (52.0%, 81.8%) and 69.2% (43.7%, 81.3%) respectively before treatment, and were 41.5% (24.5%, 80.4%) and 42.1% (15.2%, 81.0%) at the remission phase after treatment, respectively ($P < 0.05$, Table 3 and Fig. 1B and C).

5. Discussion

Despite numerous studies for allergic inflammation, the alteration of basophil activation in allergic rhinitis is still unclear and the diagnostic methods for allergic rhinitis still need development. Some studies have confirmed that basophils have antigen-presenting function, express costimulatory molecules and secrete large amounts of IL-4 [14–18]. Basophils can independently promote Th2 cell differentiation in the absence of exogenous IL-4 [19–21]. In this study, we found that patients with allergic rhinitis in acute phase had significantly higher levels of CD63 expression rate (% CCR3+ CD63+) and (% CCR3+ CD203c+) in basophils than allergic rhinitis patients in remission phase, which suggested that the alteration of basophils might be associated with patients' disease progression in allergic rhinitis. Further researches are still needed to reveal the role of basophil in allergic rhinitis and allergic inflammation.

Since basophils play an important role in allergic inflammation, BAT based on the responsiveness of basophils to specific allergens have been applied to allergen testing. A large number of clinical studies have confirmed that basophil activation status can be used for allergen diagnosis for allergic rhinitis [22–24], food allergy [25,26], drug allergy [27] and atopic dermatitis [28,29], especially when the results of routine allergen tests such as local allergic rhinitis are inconsistent with clinical symptoms [22]. Since inhalant allergens are the major allergens causing allergic rhinitis, a mixture of various inhalant allergens (pollen, tree powder, dust mites, dog dander, cat dander and mold) were used as stimulants for basophil activation test in this study. We found that patients allergic to dust mites, mold, dog dander, cat dander, pollen, tree powder and specific IgE were tested positive, which have important diagnostic value for allergic rhinitis. Considering the advantages of low volume of blood sampling (only 0.3 mL) and short test time (1–2 h), BAT may be a potential approach for evaluating the patients' disease progression.

In addition, basophil activation status has also been applied to evaluate the efficacy of the specific immunotherapy [30–32]. Since basophils are important immune cells involved in allergic inflammation, the decrease of the activated basophils after stimulation with specific allergens often indicates that the body is less sensitive

Table 3
VAS scores and basophil activation status before and after treatment of allergic rhinitis.

	n	VAS score	CCR3 ⁺ CD63 ⁺	CCR3 ⁺ CD203c ⁺
Acute phase	18	8 (6,9)	74.35 (52.0,81.8)	69.2 (43.7,81.3)
Remission phase	18	2 (0,3.2)	41.5 (24.5,80.4)	42.1 (15.2,81.0)
P		0	0.011	0.039

Statistical analyses were performed using a two-sample Wilcoxon rank-sum test with $P < 0.05$ for statistical differences; VAS: visual analogue scale.

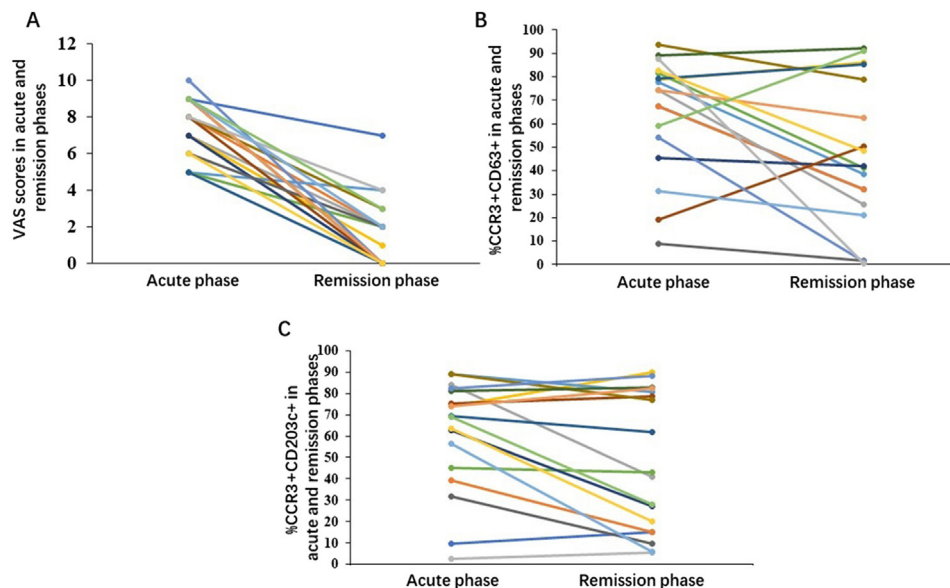


Fig. 1. A. Changes of VAS score before and after treatment of allergic rhinitis. B. Changes of CD63 expression rate (% CCR3+ CD63+) in basophils before and after treatment of allergic rhinitis. C. Changes of CD63 expression rate (% CCR3+ CD203c+) in basophils before and after treatment of allergic rhinitis.

or is tolerant to the corresponding allergens, which is often suggestive of effective control of the allergic inflammation. In this study, the degree of activated basophil was also reduced as the clinical symptoms and signs were improved significantly, suggesting that basophils can be evaluated for the efficacy of allergen therapy.

The present study also has some limitations. This research mainly concerned about the alteration of basophil activation. However, further studies are still needed to find a general standard for basophil activation in diagnosis and prognosis of allergic rhinitis.

In conclusion, our study suggests that basophils may be important immune cells involved in allergic rhinitis and basophil activation against allergen stimulation can be a biological indicator for therapeutic effects. However, the number of cases included in this study is relatively limited and the conclusions of this study need to be further confirmed in large-sample, multi-center studies.

Ethics, consent and permissions

Ethical approval was given by Shanghai Children's Medical Center Affiliated to the Medical School of Shanghai Jiaotong University and the informed consent was obtained from all patient's guardians.

Consent to publish

All of the authors have consented to publish this research.

Author's contribution

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

References

- [1] Greiner AN, Hellings PW, Rotiroli G, Scadding GK. Allergic rhinitis. *Lancet* 2011;378(9809):2112–22.
- [2] Kakli HA, Riley TD. Allergic rhinitis. *Prim Care* 2016;43(3):465–75.
- [3] Scadding GK, Kariyawasam HH, Scadding G, Mirakian R, Buckley RJ, Dixon T, et al. BSACI guideline for the diagnosis and management of allergic and non-allergic rhinitis (Revised Edition 2017; 2007). *Clin Exp Allergy* 2017;47(7):856–89.
- [4] Bernstein JA. Allergic and mixed rhinitis: diagnosis and natural evolution. *J Clin Med* 2019;8(11):2019. <http://dx.doi.org/10.3390/jcm8112019>. PMID: 31752308; PMCID: PMC6912750.
- [5] Cheng L, Chen J, Fu Q, He S, Li H, Liu Z, et al. Chinese society of allergy guidelines for diagnosis and treatment of allergic rhinitis. *Allergy Asthma Immunol Res* 2018;10(4):300–53.
- [6] Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008. *Allergy* 2008;63:8–160.
- [7] Hoyte FCL, Nelson HS. Recent advances in allergic rhinitis. *F1000Research* 2018;7. <http://dx.doi.org/10.12688/f1000research.15367.1>. F1000 Faculty Rev-1333. PMID: 30210782; PMCID: PMC6107993.
- [8] Wang X, Du K, She W, Ouyang Y, Sima Y, Liu C, et al. Recent advances in the diagnosis of allergic rhinitis. *Expert Rev Clin Immunol* 2018;14(11):957–64.
- [9] Eifan AO, Durham SR. Pathogenesis of rhinitis. *Clin Exp Allergy* 2016;46(9):1139–51.
- [10] Miyake K, Karasuyama H. Emerging roles of basophils in allergic inflammation. *Allergol Int* 2017;66(3):382–91.
- [11] Qaseem AS, Singh I, Pathan AA, Layhadi JA, Parkin R, Alexandra F, et al. A recombinant fragment of human surfactant protein D suppresses basophil activation and T-helper type 2 and B-cell responses in grass pollen-induced allergic inflammation. *Am J Respir Crit Care Med* 2017;196(12):1526–34.
- [12] MacGlashan Jr DW. Basophil activation testing. *J Allergy Clin Immunol* 2013;132(4):777–87.
- [13] Sturm EM, Kranzelbinder B, Heinemann A, Groselj-Strele A, Aberer W, Sturm GJ. CD203c-based basophil activation test in allergy diagnosis: characteristics and differences to CD63 upregulation. *Cytometry B Clin Cytom* 2010;78(5):308–18.
- [14] Cromheecke JL, Nguyen KT, Huston DP. Emerging role of human basophil biology in health and disease. *Curr Allergy Asthma Rep* 2014;14(1):408.

- [15] Siracusa MC, Comeau MR, Artis D. New insights into basophil biology: initiators, regulators and effectors of type 2 inflammation. *Ann N Y Acad Sci* 2011;1217:166.
- [16] Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin–basophil axis. *J Allergy Clin Immunol* 2014;133(5):1390–9.
- [17] Poddighe D, Mathias CB, Freyschmidt EJ, Kombe D, Caplan B, Marseglia GL, et al. Basophils are rapidly mobilized following initial aeroallergen encounter in naïve mice and provide a priming source of IL-4 in adaptive immune responses. *J Biol Regul Homeost Agents* 2014;28(1):91–103.
- [18] Olivera A, Rivera J. Paradigm shifts in mast cell and basophil biology and function: an emerging view of immune regulation in health and disease. *Methods Mol Biol* 2014;1192:3–31. http://dx.doi.org/10.1007/978-1-4939-1173-8_1. PMID: 25149480.
- [19] Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, et al. Basophils contribute to T H 2-IgE responses in vivo via IL-4 production and presentation of peptide–MHC class II complexes to CD4+ T cells. *Nat Immunol* 2009;10(7):706.
- [20] Sokol CL, Chu N-Q, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009;10(7):713.
- [21] Suurmond J, Stoop JN, Rivellese F, Bakker AM, Huizinga TWJ, Toes REM. Activation of human basophils by combined toll-like receptor-and F cε R1-triggering can promote T h2 skewing of naïve T helper cells. *Eur J Immunol* 2014;44(2):386–96.
- [22] Duarte FR, Ornelas C, Silva S, Morgado R, Pereira D, Escaleira D, et al. Contribution of in vivo and in vitro testing for the diagnosis of local allergic rhinitis. *J Investig Allergol Clin Immunol* 2019;29(1):46.
- [23] Wheatley LM, Togias A. Allergic rhinitis. *N Engl J Med* 2015;372(5):456–63.
- [24] Ogulur I, Kiykim A, Baris S, Ozen A, Yuce EG, Karakoc–Aydiner E. Basophil activation test for inhalant allergens in pediatric patients with allergic rhinitis. *Int J Pediatr Otorhinolaryngol* 2017;97:197–201.
- [25] Deng S, Yin J. Clinical utility of basophil activation test in diagnosis and predicting severity of mugwort pollen-related peach allergy. *World Allergy Organ J* 2019;12(6):100043.
- [26] Ruinemans–Koerts J, Schmidt–Hieltes Y, Jansen A, Savelkoul HFJ, Plaisier A, van Setten P. The Basophil Activation Test reduces the need for a food challenge test in children suspected of IgE-mediated cow's milk allergy. *Clin Exp Allergy* 2019;49(3):350–6.
- [27] Marraccini P, Pignatti P, D'Alcamo A, Salimbeni R, Consonni D. Basophil activation test application in drug hypersensitivity diagnosis: an empirical approach. *Int Arch Allergy Immunol* 2018;177(2):160–6.
- [28] Frezzolini A, Cadoni S, De Pità O. Usefulness of the CD63 basophil activation test in detecting Anisakis hypersensitivity in patients with chronic urticaria: diagnosis and follow-up. *Clin Exp Dermatol* 2010;35(7):765–70.
- [29] Sánchez J, Cardona R. Effect of immunotherapy on basophil activation induced by allergens in patients with atopic dermatitis. *Rev Alerg Mex* 2014;61(3):168–77.
- [30] Heffler E. Basophil activation test: a reliable biomarker for allergen immunotherapy? *Pol Arch Med Wewn* 2016;126(7–8):459–60.
- [31] Kim S-H, Kim S-H, Chung S-J, Kim J-H, Lee S-Y, Kim B-K, et al. Changes in basophil activation during immunotherapy with house dust mite and mugwort in patients with allergic rhinitis. *Asia Pacific Allergy* 2018;8(1). <http://dx.doi.org/10.5415/apallergy.2018.8.e6>. PMID: 29423373; PMCID: PMC5796966.
- [32] de la Varga Martínez R, Fernández DG, Áñez GA, Fernández AF, García JAA, Varo FM. Use of the basophil activation test in monitoring clinical tolerance after desensitization to brentuximab vedotin. *Ann Allergy Asthma Immunol* 2017;118(6):745–7.