Gold standard diagnostic algorithm for the differential diagnosis of local allergic rhinitis

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Abstract

Local allergic rhinitis, defined as a localized allergic response of the nasal mucosa in the absence of systemic atopy, poses a considerable clinical issue due to its prevalence. The gold standard in local allergic rhinitis diagnostics is primarily the patient's history taking and nasal allergen provocation testing or, alternatively, the basophil activation test, described as "an allergic reaction in a test tube".

Key words: local allergic reaction, nasal allergen provocation test, basophil activation test.

Introduction

Rhinitis is an inflammation of the nasal mucous membrane which manifests itself clinically by sneezing, itching, watery discharge and a feeling of nasal cavity congestion. Moreover, these symptoms must last for over 1 h a day for many days of the year [1]. Rhinitis is an extremely heterogeneous group of diseases with respect to aetiology. We can distinguish between infectious and non-infectious rhinitis. Non-infectious rhinitis can be divided into allergic rhinitis (AR) and non-allergic rhinitis (NAR). The common feature of non-allergic rhinitis is the absence of atopy. In this group we can identify: drug-related rhinitis, non-allergic rhinopathy, non-allergic rhinitis with eosinophilia (NARES), senile rhinitis, atrophic rhinitis, gustatory rhinitis and hormonal rhinitis [1].

Allergic rhinitis (AR) is an IgE-dependent inflammation of the nasal mucosa resulting from exposure to an allergen and is the most common form of non-infectious rhinitis. It affects about 35% of the general population, and the incidence of this disease is constantly increasing [1]. According to present knowledge, allergic rhinitis occurs in two forms: classic AR as a manifestation of a systemic allergy with systemic atopy and positive results of skin prick tests and/or sIgE tests and local AR (local allergic rhinitis – LAR) as a local allergic reaction (specific AR phenotype), affecting only the nasal mucosa without systemic atopy, with negative results of skin prick tests and/or sIgE tests [2]. The first reports of a local allergic reaction go back to the 1970s. In 1975, Huggins and Bostoff demonstrated local production of sIgE after a nasal provocation test was performed on patients with symptoms of rhinitis and negative results of skin prick tests [3]. In 2003, Powe et al. proposed a new term, "entopy", which refers to a local allergic reaction as opposed to a systemic reaction – atopy [4]. Unquestionably, the greatest contribution to understanding the essence of the local allergic reaction and pathomechanism, diagnostic investigation and treatment of local rhinitis was brought by Spanish scientists led by Carmen Róndon [5]. In their prospective study, lasting 10 years, the above mentioned authors showed a low conversion rate of the allergic local reaction to systemic atopy (positive results of skin prick tests and/or sIgE tests) in both LAR and control groups, 9.7% vs. 7.8%, respectively, proving a distinct and specific picture of this disease [6].

Definition

The term *local allergic rhinitis* (LAR) was proposed by Carmen Róndon in 2010 [5]. Local allergic rhinitis is a specific clinical phenotype of rhinitis. Its symptoms are similar to those of AR with a local inflammatory response mediated by Th2 lymphocytes and the production of allergen specific IgE antibodies in the nasal mucosa. The absence of sIgE on the mast cells of the skin as well as in the serum and a positive result of a nasal allergen provocation test are also very important. According to present knowledge, LAR cannot be treated as an early manifestation of classic AR [2, 6, 7].

Epidemiology

The epidemiological data on LAR are quite diverse. A number of studies by Spanish authors have shown the incidence of LAR in 50–75% of the population with rhinitis symptoms, without a confirmed systemic atopy [2, 7]. In turn, studies from Asia suggest a lower (less than 20%) incidence of LAR compared to Western countries [8, 9]. In 2017, Polish scientists, Krajewska-Wojtys *et al.* in a group of 84 adult patients with chronic rhinitis and negative results of skin prick tests and sIgE tests diagnosed LAR in 21 of them (25%). In all patients a nasal allergen provocation test was performed and sIgE was determined in nasal lavage fluid. Most LAR patients were allergic to *Dermatophagoides pteronyssinus* – 19 (22.6%) [10]. In another multicentre Polish study in 2019, Bożek *et al.*, in a group of 621 patients with chronic rhinitis found LAR in 109 of them (17.6%), AR in 251 of them (40.4%) and NAR in 261 of them (42%). In the group with LAR, young, non-smoking patients, allergic mainly to house dust mites and grass pollen were dominant [11].

Pathomechanism

LAR immunopathology is very similar to AR immunopathology, but the main difference is the local nature of the allergic reaction [1, 2]. Local allergic rhinitis is characterized by an inflammatory response mediated by Th2 lymphocytes and the production of specific IgE antibodies and other inflammatory mediators in the nasal mucosa. Exposure to allergens causes their presentation by dendritic cells, macrophages, mast cells and endothelial cells to Th2 lymphocytes, which leads to stimulation of these cells. Stimulation of Th2 lymphocytes leads to production of numerous cytokines, including IL-4, IL-5 and IL-13. Cytokines stimulate B lymphocytes to produce specific IgE antibodies. Th2 lymphocytes produce chemokines (RANTES, eotaxin 1, eotaxin 2, MCP1,-3,-4) which are responsible for the mobilisation of eosinophils and their migration to the subepithelial layer. An increased IgE production facilitates their connection to receptors on the surface of mast cells. The combination of two allergen molecules with one IgE antibody molecule causes de-

granulation of the mast cell. Within a few seconds/minutes after contact with the allergen, the early phase of the allergic reaction begins and usually lasts 60–90 min. Degranulation of mast cells leads to the release of many preformed mediators, especially histamines, tryptases, chymases and others. The degranulation of the mast cell also leads to the *de novo* formation of many mediators, such as cysteine, leukotrienes, leukotriene B4, prostaglandin D2 and platelet activating factor (PAF). Cytokines are also released in the early phase of TNF, IL-6 and IL-1, and in the late phase IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, GM-GSF synthesis takes place. The late phase starts a few dozen minutes after the exposure and lasts several hours. The essential cells of the late phase are basophils, eosinophils and T lymphocytes and mediators they produce. Most of the IL-4s in the late phase come from basophils, they are also involved in the switching of antibody classes and are responsible for local histamine production. Eosinophils are a source of ECP, EPO and MBP [1, 2, 7].

The clinical picture

LAR patients present clinical symptoms typical of rhinitis, i.e. sneezing, itching, watery mucus discharge from the nose and nasal congestion [1]. These symptoms are often accompanied by symptoms from the lower airways and conjunctivitis. In clinical history it is often possible to establish a link between these symptoms and exposure to particular allergens [2]. Based on many years of observation, a typical clinical profile of a patient with LAR has been established. It is usually a young, non-smoking woman, resident of a large agglomeration, with moderate to severe rhinitis symptoms. Patients with LAR often have a family history of atopic diseases [2, 7].

Differentiation and concomitant diseases

Patients with AR and LAR have many common clinical and demographic features, but in patients with LAR, despite the typical clinical picture, the results of skin prick tests and sIgE are negative [2]. In both diseases, on the other hand, a nasal allergen provocation test is positive. LAR should also be differentiated from non-allergic rhinitis [1, 2, 7]. The most important differentiating elements are that a patient with LAR usually has more severe symptoms of rhinitis, is a non-smoker, has a positive family history of atopy, is a female and is younger [2]. However, the most important conclusive test for the diagnosis is a positive result of an allergen provocation test in patients with LAR [2, 7]. Other nasal and paranasal sinus diseases, such as chronic paranasal sinusitis with and without polyps, hypertrophy of the pharyngeal tonsil, a different anatomical structure of the lateral wall of the nose, or a deformation of the nasal septum, should also be considered in the differential diagnosis. This is usually determined by

an ENT examination and diagnostic imaging (CT, MRI). It is also important to remember about systemic diseases with nasal symptoms, such as primary ciliary dyskinesia, cystic fibrosis, sarcoidosis, Wegener's granulomatosis and many others [2]. Many studies highlight the relationship between LAR and asthma (A) and about 30% of patients with this disease declare symptoms typical for A [2, 7]. In their study evaluating the occurrence of bronchial asthma in patients with LAR as a consequence of house dust mite allergy, Campo et al. found a positive result of a bronchial provocation test with methacholine in 50% of LAR patients, in 83.3% of AR patients and in 57.9% of NAR patients. A bronchial provocation test was positive in 28% of LAR patients, 83% of AR patients and in none of NAR patients. In a methacholine control provocation test performed 24 h after an allergen provocation test, a significant deterioration of spirometric parameters as well as an increase in inflammatory mediators (ECP, tryptase and sIgE) in induced sputum were found in all patients with positive results of the allergen provocation test. The results allowed for the hypothesis of a local allergic reaction also in the bronchi [12]. In their study on the Polish population, Bożek et al. found the occurrence of bronchial asthma at a similar level in groups of patients with LAR (35%) and AR (38%) and significantly less frequently in the group of patients with NAR (16%) [11]. Patients with LAR often report symptoms of conjunctivitis during natural exposure to allergens and during an allergen provocation test. Eye symptoms are more frequent for pollen allergens than for house dust mites. However, it is still not entirely clear whether these symptoms are related to the actual allergic reaction or are an expression of the nasal-ocular reflex after nasal exposure [2, 7].

Diagnostic investigation as a basis for diagnosis

LAR diagnosis is based on the past medical history and a positive nasal allergen provocation test (NAPT) (Figure 1). Alternative assessments include the basophil activation test (BAT), which is called "an allergic reaction in a test tube", or testing for sIgE in nasal lavage fluid. The latter two tests are less commonly used in LAR diagnostics than the NAPT. In case of contraindications to a NAPT, the BAT is believed to be a valid alternative in qualifying the patient for further treatment, though confirming this requires further studies.

Medical history

A number of LAR patients develop the condition in their early childhood and have a family history positive for atopy. Clinical manifestations of LAR are similar to those of classic AR: itching, sneezing, rhinorrhoea, and persistent nasal congestion. LAR is diagnosed based on the combination of a history of characteristic AR symptoms and diagnostic test results. History-taking is a crucial stage of the diagnostic process as it provides a number of important patient data and determines further actions to be taken [4]. History should include the duration, character, severity, and possible seasonality of the presenting symptoms; the effect the symptoms have on the patient's quality of life (occupation, learning, sleep,
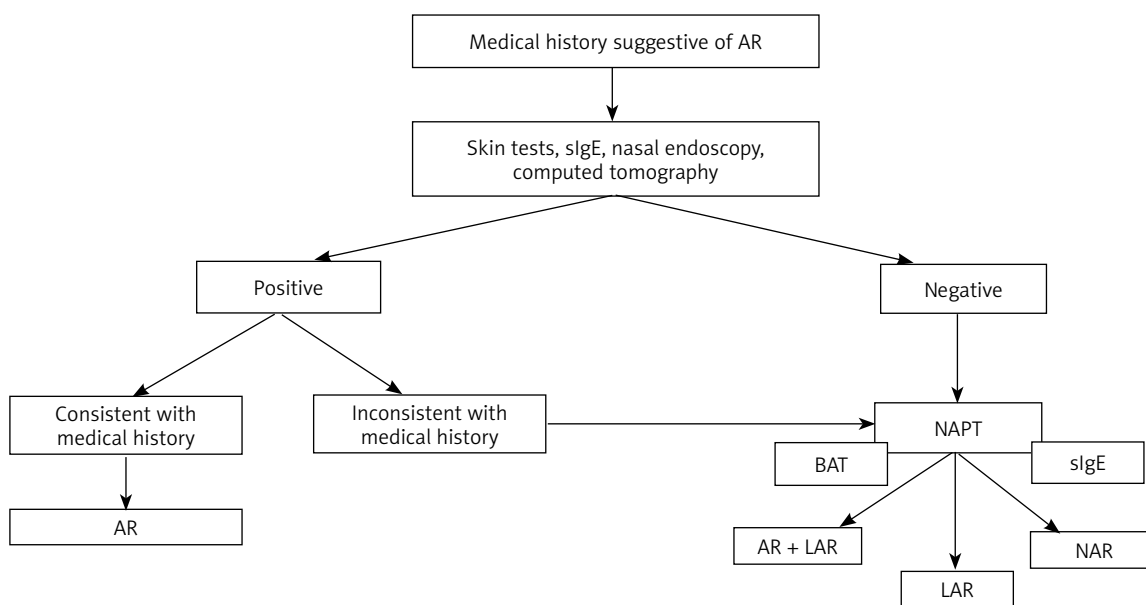
AR – allergic rhinitis, BAT – basophil activation test, NAPT – nasal allergen provocation test, NAR – non-allergic rhinitis, LAR – local allergic rhinitis, sIgE – specific IgE.

Figure 1. Diagnostic algorithm for local allergic reactions, based on Campo et al. and Rondon et al. [2, 5, 6]

exercise); possible family history of atopy; concomitant symptoms, especially cough, wheezing, dyspnoea, watery and itchy eyes, and exposure to potential allergens.

Physical examination

A physical examination involves a thorough medical assessment with special attention paid to an ENT examination. In order to examine the nose, an anterior rhinoscopy and an endoscopic examination of the nose and nasopharynx are performed. An anterior rhinoscopy is used to assess the appearance of the nasal mucosa, the nature of the discharge, the presence of possible pathological forms and structural changes (deviation of the nasal septum). An endoscopic examination allows for a very precise assessment of those structures which are not accessible through a rhinoscope: elements of the lateral wall of the nasal cavity, the nasal septum and nasopharynx [1].

Nasal allergen provocation tests

The NAPT is conducted in a controlled (outpatient or inpatient) setting, which "recreates the natural upper-airway response to a locally applied allergen" [7]. Among the methods used in rhinology and allergy diagnosis, the NAPT is characterized by high sensitivity, specificity [7, 8], and a relatively good safety profile [13]. Allergic response assessment via objective (acoustic rhinometry, rhinomanometry, peak nasal inspiratory flow (PNIF) measurement) and subjective measures [9, 10] provides valuable information on the extent of patients' reactivity to allergens commonly found in their environment. Based on international expertise, the European Academy of Allergy and Clinical Immunology (EAACI) precisely defines a clearly positive NAPT result (for a rhinomanometry flow decrease of ≥ 40% at 150 Pa, PNIF flow decrease of ≥ 40%, and acoustic rhinometry CSA decrease of ≥ 40%). According to the consensus, apart from employing objective measures, also subjective nasal symptoms should be assessed as part of the NAPT. When a visual analog scale (VAS) is used, a score of ≥ 55 mm is considered to be a clearly positive result [9]. The NAPT is well tolerated by 99.9% of all LAR patients [3, 12]. The fact that LAR has been shown to co-exist with other conditions (including local allergic asthma and conjunctivitis [3]) warrants expanding diagnostic measures to include assessing the lower airway function (spirometry, exhaled nitric oxide) and confirming the presence of subjective ocular and bronchial symptoms.

The NAPT is one of few allergy diagnostic methods that recreate the body's natural response to an allergen. The observed cellular-level changes resemble those taking place during a local anaphylactic reaction. The early phase of the allergic reaction in response to thee NAPT is characterized by such symptoms as itching in the nose, watery discharge from the nose, and nasal congestion

[11]. The late phase of the allergic reaction (which takes place 4–48 h after local allergen application) is characterized by a potential risk of an allergic response from the lower airways both in patients with AR and LAR (especially those with concomitant bronchial asthma). Studies show that 60% of the LAR patients who are allergic to allergens commonly found in their environment and who are also diagnosed with bronchial asthma experience symptoms from the lower airways in response to nasal allergen provocation [13, 14]. Nearly 50% of the evaluated LAR population who underwent a methacholine provocation test manifested a nonspecific bronchial response, whereas specific (bronchial) provocation yielded a positive result in 28.8% of patients [12].

The scope of experimental studies allows for measuring sIgE levels in nasal lavage fluid alongside with or independently from the NAPT. Importantly, the method of measuring sIgE levels has a lower sensitivity than the NAPT (as only 20–43% of patients with LAR manifested increased sIgE levels in nasal lavage fluid) [12, 13]. A study by Meng *et al.*, published this year and involving patients with LAR, demonstrated a high sensitivity (91.7%), specificity (95.1%), and predictive value (of both positive (78.6%) and negative (98.3%) results) of measuring sIgE levels in nasal lavage fluid. The diagnostic accuracy of measuring sIgE levels in nasal lavage fluid in this group of patients was 94.5% [14].

Moreover, the level of sIgE against molecules from different allergen sources has been attempted to be tested recently using microarray technologies. A study by Berings *et al.* demonstrated that measuring sIgE levels in nasal secretions (in patients with AR) showed 100% specificity and 70% sensitivity compared with that of serum sIgE levels. Moreover, sIgE to 15 house dust mite allergens, including 13 *Dermatophagoides pteronyssinus* molecules (i.e. nDer p 1, rDer p 2, rDer p 4, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 14, rDer p 15, rDer p 18, rDer p 21, rDer p 23, and clone 16) and two *Dermatophagoides farinae* molecules (i.e. nDer f 1 and rDer f 2) were measured with ImmunoCAP ISAC in serum (cutoff ≥ 0.10 ImmunoCAP Standardized Units ISU/ml). The presence of sIgE to at least one of the major allergen molecules (nDer p 1, nDer f 1, rDer p 2, rDer f 2, and/or rDer p23) in nasal secretions predicted with high specificity (100%) and sensitivity (90%) both the patient's allergic status and the serum sIgE levels [15].

Basophil activation test

The BAT is a cytometric study assessing basophil activation following stimulation with an allergen. Despite the fact that basophil activation has raised considerable scientific interest, the methods of performing the test still vary widely between research centres, and there are problems that are still unresolved. Some members of the EAACI formed the European Interest Group for the

Evaluation of the BAT in clinical routine (EuroBAT). The researchers use commercially available test kits or introduce their own protocols. The BAT may be performed with whole blood or isolated cells [16, 17]. Cell isolation is conducted via density-gradient centrifugation. On the other hand, the use of whole blood is easier from a technical perspective, and the fact that it contains all its natural components may better reflect the physiological and pathological conditions found *in vivo* [18]. In order to react immediately following stimulation, basophils require an optimal temperature, adequate incubation time, and appropriate dilution-buffer composition. Some authors postulate that short preincubation with IL-3 may increase the sensitivity of CD63-based tests. However, since IL-3 increases CD203c expression on inactive basophils and reduces the sensitivity of CD203c-based BATs, its use is controversial [19]. An accurate interpretation of BAT results requires the use of appropriate negative and positive controls. The negative control shows the spontaneous expression of activation markers. This is done by incubating basophils in the washing solution. The number of activated basophils in the negative control rarely exceeds 5% (multi-centre study data indicate 0–5% basophil stimulation in 79.9% of subjects; 5–10% in 13.6% of subjects; and > 10% in 6.5% of subjects) [20]. The specific, positive control is based on anti-IgE antibodies; alternatively, on monoclonal anti-FcεRI antibodies. In the case of a lack of response to anti-IgE or anti-FcεRI antibodies, IgE-independent stimuli, such as N-formyl-methionyl-leucyl-phenylalanine (fMLP), are used. A properly conducted positive stimulation test should yield > 10% of activated basophils. A nonspecific positive control with fMLP may be used to determine cell viability [21]. When interpreting BAT results, it is important to remember that there are patients whose basophils do not become activated and do not secrete mediators following stimulation with the positive control. False-negative BAT results may be also explained by a transient basophil nonresponsiveness due to a recent exposure to the allergen. As a result of using up their sIgE during an episode of an acute allergic reaction, these patients may have temporarily reduced levels of circulating and membrane-bound sIgE. Therefore, the BAT should be postponed by 4–6 weeks following an acute allergic reaction. Moreover, false-negative results may be associated with technical problems, such as incorrect test-tube storage or transportation, or the use of inappropriately selected stimulating allergens. The allergens used for these tests should effectively activate basophils and contain no cytotoxic agents (additives, preservatives), or nonspecifically stimulating substances (endotoxins, lectins) [22]. The BAT is an "allergic reaction in a test tube". Essentially, the test uses flow cytometry to quantify the expression of activation markers (including CD63, CD203c) on basophil surface, in whole blood, following stimulation with an allergen. Other than by/Apart from measuring cell surface marker expression,

basophil activation can be also assessed based on the phosphorylation of certain intracellular molecules, such as the p38 mitogen-activated protein kinase (MAPK) or signal transducer and activator of transcription (STAT) 5, which are part of the signalling cascade downstream of IgE and its high-affinity receptor [23, 24]. Other methods of assessing basophil activation (using cytometry by time-of-flight (CyTOF) [25] and fluorochrome-labelled avidin [26]) have also been suggested.

Positive BAT results have been reported in nearly 50–53% of patients with LAR [3], which proves that this technique, as well as that of measuring sIgE levels in nasal lavage fluid, is only an additional investigation to the NAPT in LAR diagnostics [27–32].

The treatment

Since there is a close clinical relationship and a similar pathomechanism, the treatment principles of LAR are similar to those of AR. Educating patients about the essence of their disease and how to avoid the causative factor and methods to reduce exposure to allergens is of great importance. Pharmacological treatment involves the use of intranasal corticosteroids and second-generation oral antihistamines [2, 7]. The only cause-based method of treating allergies is allergen-specific immunotherapy, which reduces the risk of developing bronchial asthma and inhibits the development of new allergies [1]. Róndon and colleagues presented encouraging results on specific immunotherapy for the treatment of LAR in patients allergic to grass and house dust mites based on randomised double-blind placebo-controlled trials [33, 34]. Polish authors Bozek and colleagues also presented the results of treatment of LAR patients suffering from birch pollen allergy, using specific immunotherapy, with good clinical effects [35].

Summary

Due to the extent of LAR comorbidities and their consequences, there is an urgent need to implement the gold standard diagnostic algorithm for the differential diagnosis of local allergic rhinitis, which is based largely on history taking and nasal provocation testing.

Conflict of interest

The authors declare no conflict of interest.

References

1. Samoliński B, Arcimowicz M. Polskie Standardy Leczenia Nieżytów Nosa (PoSLeNN). Warszawa 2014.
2. Campo P, Eguiluz-Gracia I, Bogas G, et al. Local allergic rhinitis: implications for management. Clin Exp Allergy 2019; 49: 6-16.

3. Huggins KG, Brostoff J. Local production of specific IgE antibodies In allergic-rhinitis patients with negative skin tests. Lancet 1975; 2: 148-50.
4. Powe DG, Jagger C, Kleinjan A, et al. Entopy: localized mucosal allergic disease in the absence of systemic responses for atopy. Clin Exp Allergy 2003; 33: 1374-9.
5. Rondon C, Canto G, Blanca M. Local allergic rhinitis a new entity, characterization and further studies. Curr Opin Allergy Clin Immunol 2010; 10: 1-7.
6. Rondon C, Campo P, Equiluz-Gracia I, et al. Local allergic rhinitis is an independent rhinitis phenotype: the results of a 10-year follow-up study. Allergy 2018; 73: 470-8.
7. Eguiluz-Gracia I, Perez-Sanchez N, Bogas G, et al. How to diagnose and treat local allergic rhinitis: a challenge for clinicians? J Clin Med 2019; 8: 1062.
8. Cheng KJ, Xu YY, Liu HY, et al. Serum eosinophil cationic protein level in Chinese subjects with nonallergic and local allergic rhinitis abd its relation to the severity of disease. Am J Rhinol Allergy 2013; 27: 8-12.
9. Chang GU, Jang TY, Kim KS, et al. Nonspecific hyperreactivity and localized allergy: cause of discrepancy between skin prick and nasal provocation test. Otolaryngol Nead Neck Surg 2014; 150: 194-200.
10. Krajewska-Wojtys A, Jarzab J, Zawadzinska K et al. Local allergic rhinitis In adult patients with chronic nasal symptoms. Int Arch Allergy Immunol 2017; 173: 165-70.
11. Bożek A, Scierski W, Ignasiak B, et al. The prevalence and characteristics of local allergic rhinitis in Poland. Rhinology 2019; 57: 213-8.
12. Campo P, Eguiluz-Gracia I, Plaza-Seron MC et al. Bronchial asthma triggered by house dust mites in patients with local allergic rhinitis. Allergy 2019; 74: 1502-10.
13. Samoliński B, Rapiejko P, Krzych-Fałta E, et al. Definicja donosowej próby prowokacyjnej i donosowej próby prowokacyjne z alergenem, klasyfikacja. Adv Dermatol Alergol 2010; 27: 166-9.
14. Mazurek H, Modrzyński M. Test donosowej próby prowokacyjnej z alergenem na tle innych badań diagnostycznych. Adv Dermatol Alergol 2010; 27: 193-5.
15. Augé J, Vent J, Agache I, et al. EAACI Position paper on the standardization of nasal allergen challenges. Allergy 2018; 73: 1597-608.
16. Pepper AN, Ledford DK. Nasal and ocular challenges J Allergy Clin Immunol 2018; 141: 1570-7.
17. Samoliński B, Rapiejko P. Odpowiedź wczesna po donosowej próbie prowokacyjnej z alergenem. Adv Dermatol Alergol 2010; 27: 170-2.
18. Eguiluz-Gracia I, Testera-Montes A, González M, et al. Safety and reproducibility of nasal allergen challenge. Allergy 2019; 74: 1125-34.
19. Naclerio RM, Proud D, Peters SP, et al. Inflammatory mediators in nasal secretions during induced rhinitis. Clin Allergy 1986; 16: 101-10.
20. Meng Y, Wang Y, Wang K, et al. Specific immunoglobulin E in nasal secretions for the diagnosis of local allergic rhinitis. Rhinology 2019; 57: 313-20.
21. Berings M, Arasi S, Ruyck ND, et al. Reliable mite-specific IgE testing in nasal secretions by means of allergen microarray. J Allergy Clin Immunol 2017; 140: 301-8.
22. Gelardi M, Gugliemi A, Iannuzzi L, et al. Local allergic rhinitis: entopy or spontaneus response? World Allergy Organ J 2016; 9: 39.
23. Ebo DG, Bridts CH, Hagendorens MM, et al. Basophil activation test by flow cytometry: present and future applications in allergology. Cytometry B Clin Cytom 2008; 74B: 201-10.
24. De Weck AL, Gamboa P, Sanz ML. Comparison of two commercial basophil activation cytofluorometric tests for the diagnosis of allergies and pseudoallergies. EAACI Paris 2003; 369-70.
25. Leśniak M, Dyga W, Porębski G, et al. Basophil activation test-a practical approach to diagnosis of common respiratory allergy. Przegl Lek 2015; 72: 725-30.
26. Piotrowicz-Wójcik K, Dyga W, Obtułowicz K. Test aktywacji bazofilów (CD63+): porównanie metod BAZOTEST Orpegen oraz Flow2CAST Bühlmann Laboratories. Alergol Immunol 2010; 7: 16-8.
27. Hemmings O, Kwok M, McKendry R, et al. Basophil activation test: old and new applications in allergy. Curr Allergy Asthma Rep 2018; 18: 77.
28. Wolanczyk-Medrala A, Gogolewski G, Liebhart J, et al. A new variant of the basophil activation test for allergen-induced basophil CD63 upregulation. The effect of cetirizine. J Investig Allergol Clin Immunol 2009; 19: 465-73.
29. Ebo DG, Dombrecht EJ, Bridts CH, Aerts NE, de Clerck LS, Stevens WJ. Combined analysis of intracellular signalling and immunophenotype of human peripheral blood basophils by flow cytometry: a proof of concept. Clin Exp Allergy 2007; 37: 1668-75.
30. Verweij MM, Sabato V, Nullens S, et al. STAT5 in human basophils: IL-3 is required for its FcepsilonRI-mediated phosphorylation. Cytometry B Clin Cytom 2012; 82: 101-6.
31. Mukai K, Gaudenzio N, Gupta S, et al. Assessing basophil activation by using flow cytometry and mass cytometry in blood stored 24 hours before analysis. J Allergy Clin Immunol 2017; 139: 889-99e11.
32. Mukai K, Chinthrajah RS, Nadeau KC, et al. A new fluorescent-avidin-based method for quantifying basophil activation in whole blood. J Allergy Clin Immunol 2017; 140: 1202-6.
33. Rondon C, Blanca-Lopez N, Campo P, et al. Specific immunotherapy in local allergic rhinitis: a randomized, double-blind placebo-controlled trial with Phleum pretense subcutaneous allergen immunotherapy. Allergy 2018; 73: 905-15.
34. Rondon C, Campo P, Salas M, et al. Efficacy and safety of D. pteronyssinus immunotherapy in local allergic rhinitis: a double-blind placebo-controlled clinical trial. Allergy 2016; 71: 1057-61.
35. Bozek A, KolodziejczykK, Jarzab J. Efficacy and safety of birch pollen immunotherapy for local allergic rhinitis. Ann Allergy Asthma Immunol 2018; 120: 53.