Original paper

Allergic and nonallergic rhinitis in schoolchildren. Part II. Relationship to spirometric parameters, bronchial hyperresponsiveness and biomarkers of allergic inflammation

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Abstract

Introduction: In recent years, with the increasing prevalence of allergic rhinitis (AR), a higher incidence of nonallergic rhinitis (NAR) has been observed.
Aim: The aim of the study was the comparison of pulmonary function tests and the degree of bronchial hyperresponsiveness (BHR) in children with AR and NAR, evaluation of the biomarker for clinical AR or BHR diagnosis.
Material and methods: Pulmonary function tests and BHR in the exercise tests were done in 139 schoolchildren (8-14 years of age) with symptoms of non-infectious rhinitis. The analyzed biomarkers included exhaled nitric oxide (FeNO), total IgE (tIgE), serum IL-4, serum tumour necrosis factor α (TNF-α), absolute eosinophils count in blood (Eos). Positive prick tests with 10 aeroallergens identified children with AR.
Results: Spirometric parameters and values of BHR were not different in AR (n = 73) and NAR (n = 66) groups. The risk of BHR was higher in children with AR than those with NAR (OR = 2.768; p = 0.051). In the logistic regression analysis, none of the analyzed biomarkers was related to BHR. Differences in the area under the ROC curve (AUC) for: tIgE and FeNO (AUC_{tIgE}-AUC_{FeNO} = 0.102; p = 0.042), FeNO and Eos (AUC_{FeNO}-AUC_{Eos} = 0.07; p = 0.219), tIgE and Eos (AUC_{tIgE}-AUC_{Eos} = 0.172; p = 0.002) showed that measurements of tIgE level were the best diagnostic tool for AR (sensitivity = 78.9%, specificity = 79.4% for tIgE = 68 kU/l).
Conclusions: There were no differences in pulmonary function test parameters between groups of children with AR and NAR. Bronchial hyperresponsiveness was more often observed in AR than in NAR. Any biomarker of allergic inflammation predicts BHR in children with rhinitis. The accuracy of diagnostic AR testing with tIgE measurement (cut-off level = 68 kU/l) was moderately good in schoolchildren.

Key words: allergic rhinitis, nonallergic rhinitis, pulmonary function tests, biomarkers.

Introduction

In the last decades, an increased prevalence of allergic diseases in children has been observed, including allergic rhinitis. Moreover, accumulation of clinical and epidemiological data indicates that incidence of chronic rhinitis without any allergic background has been also increasing [1, 2]. Nowadays, non-infectious rhinitis could be classified into the following groups: 1) allergic rhinitis (AR) – where IgE-mediated mechanisms are involved (positive skin prick tests, increased specific IgE in the serum to the specified allergens); 2) nonallergic rhinitis (NAR) – the systemic IgE-related mechanisms is not possible to determine as it can be done in AR. However, there are increasing experimental data indicating that local allergic inflammation restricted to nasal mucosa could be shown [3]; 3) *rhinosinusitis* – a clinical situation, in which an infectious agent causes a chronic inflammatory process of the nose and the paranasal sinuses [4, 5]; 4) other uncommon conditions, especially in children, related to exposure to noxious agents, some non-steroid anti-inflam-

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matory drugs, structural abnormalities of the nasopharyngeal cavity or systemic diseases (immunodeficiency, cystic fibrosis, primary cilia dyskinesia and others) [6].

Presently, there are no laboratory tests to confirm that a patient suffers from NAR, and this condition is defined by exclusion, i.e. absence of the allergen specific IgE antibodies and absence of the active infectious process. Recognition of limited knowledge in the NAR area has contributed to further research perspectives of clinical and epidemiological studies, which was formulated by GA^{2}LEN (Global Allergy and Asthma European Network) [1].

Aim

The aim of the present study, complementary to previous one [7], was to characterize and compare the pulmonary function tests and some biomarkers of allergic inflammatory processes in schoolchildren with AR and NAR and to determine which of these parameters would be the most suitable for differentiation and clinical diagnosis of these two types of rhinitis.

Material and methods

Study population

Children were enrolled to the study through the Allergy Preventive Program held in Krakow, implemented by the Outpatient Department of Pulmonology and Allergy of the Polish-American Children's Hospital. Invitations to the program were distributed by local media. After a short phone survey of parents, children who fulfilled inclusion criteria were invited to visit the outpatient department. During the first study visit, pre-enrollment conditions obtained by phone were confirmed and a more precise interview was carried out. The following inclusion criteria were employed: 1) 8-14 years of age; 2) living within the city limits of Krakow; 3) chronic, permanent nasal symptoms in the last year. The exclusion criteria were: 1) treatment with antihistaminic medicines in the last 4 weeks; 2) treatment with nasal aerosols in the last 4 weeks; 3) treatment with antiasthmatic medicines (inhaled or systemic) in the last 4 weeks; 4) established diagnosis and treatment of AR in the past; 5) signs of *rhinosinusitis* in the past and/or during the present visit (anterior rhinoscopic examinations were done); 6) established diagnosis of asthma or other chronic respiratory tract disorders or other systemic diseases; 7) acute infection of the respiratory system in the last 4 weeks. The visits were scheduled for September and October.

The study was approved by the Bioethical Committee of Jagiellonian University. All the parents and children above 12 years of age gave their written informed consent.

Clinical questionnaire-based interviews

Presentation and qualification of clinical symptoms were identified with a modified ISAAC questionnaire for Phase II of that study. The questionnaires were filled out by children and their parents under the investigator's supervision. In the module of nasal symptoms, questions asked concerned the type of nasal symptoms apart from a cold or the flu in the last year (verification of answers obtained during phone questioning), their duration (above 4 weeks and longer than 4 days in a week) for rhinitis classification purposes (intermittent, persistent) according to the ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines [6].

Skin prick tests

Sensitization was assessed by skin prick tests (SPT) performed in a typical manner, using the following 10 allergens (Allergopharma, Germany): indoor allergens – house dust mites (*D. pteronyssimus*, *D. farine*), dog, cat, molds (*Alternaria*, *Aspergillus*, *Cladosporium*), outdoor allergens – grasses, trees, weeds. The reaction to each of the allergens was regarded positive if the mean wheal diameter was at least 3 mm. Atopy was defined as a positive SPT to at least one of the aeroallergens.

Definitions of diagnosis

Allergic rhinitis was diagnosed if atopy and nasal symptoms (sneezing, itching, rhinorhea, nasal obstruction) were present, apart from viral infections, in the last year. Nonallergic rhinitis was diagnosed if the condition of rhinitis was fulfilled, but atopy was not present.

According to the ARIA classification, chronic rhinitis was established when duration of rhinitis was more than 4 days per week and longer than 4 weeks in the last year. Intermittent rhinitis was diagnosed when duration of symptoms did not fulfill conditions of chronic rhinitis.

Lung function tests

The lung function tests were performed with the spirometer – Lungtest 1000 (MES, Kraków, Poland) according to ERS/ATS [8] standards. Three acceptable forced exhalation attempts, lasting more than 3 s, with distinguished flow plateau at the end of exhalation allowed for choosing the best parameters: FEV_1, FVC, $FEF_{25-75\%}$, FEV_1/FVC and computing the percentage of the predicted value using the equations provided in the spirometer software [9]. The value of FEV_1 below 80% of the predicted value was an indication to perform the bronchodilator-response tests with 200 µg of salbutamol given by spacer. A relative increase of FEV_1 above 12% and an absolute increase of more than 200 ml indicated positivity of the test.

Bronchial hyperresponsiveness

The exercise challenge test was done in concordance with published guidelines for bronchial hyperresponsiveness (BHR) estimation [10]. In brief, all the children performed baseline spirometry before the challenge test. Dur-

ing the test, the heart rate and SpO_2 were monitored with a pulse oxymeter. Adequate child cooperation and its FEV_1 above 80% of the predicted value were the conditions for qualification to the challenge test. The exercise test was performed using a treadmill and the effort was increased by the slope and speed during 6 min of running and adjusted to obtain 80-90% of the maximal predicted heart rate (calculated as 220 – age in years) in the last 4 min of the test. Appropriate conditions of ventilation were maintained by a nose clip, room air temperature of 19-22°C and relative air humidity below 55%. Spirometry with FEV_1 measurement was performed 1, 5, 10, 15 and 20 min after exercising. Bronchial hyperresponsiveness was recognized when a relative decrease of FEV_1 after the test was no less than 10% ($\Delta FEV_1 \geq 10\%$). The children having baseline FEV_1 below 80% and positive bronchodilator-response test after salbutamol were regarded as these with BHR and the value ΔFEV_1 of 25% was arbitrarily designated for them for further statistical analyses.

Biomarkers of allergic process

The following biomarkers were selected for identification of allergic inflammation: exhaled nitric oxide (FeNO), serum total IgE (tIgE), absolute eosinophils count of the peripheral blood (Eos), serum interleukin 4 (IL-4) and tumour necrosis factor α (TNF-α).

Measurement of exhaled nitric oxide (FeNO) were performed with NIOX MINO® (Airway Inflammation Monitor (NIOX MINO); Aerocrine AB, Solna, Sweden) following international guidelines and the manufacturer's instructions, maintaining 50 ±5 ml/s air flow during exhalation [11].

The levels of tIgE were measured using the immunofluorometric method (ImmunoCAP, Phadia AB, Sweden) with the assay sensitivity range of 2-5000 kU/l. Serum levels of IL-4 and TNF-α were estimated by ELISA with Quantikine®HS kits (R&D Systems, Inc., USA) following the manufacturer's instructions and with the sensitivity measurements of 0.03-0.22 pg/ml and 0.038-0.191 pg/ml, respectively. The absolute eosinophils counts were calculated according to standard hospital laboratory methods.

Statistical analysis

Differences in continuous data were estimated with the parametric tests with or without logarithmic transformation of variables and presented as geometric or arithmetic means with 95% confidence interval (95% CI) or standard deviation (SD), as appropriate. In the case of rejection of normality distribution after logarithmic transformation, the Mann-Whitney test was used and differences between data were displayed by the median value of the variable with 95% CI. The relationship of categorical data was estimated by the χ^2 test and in the case of significance level of this test, the odds ratio (OR) with 95% CI was calculated. The ROC (Receiver Operating Characteristic) curves were used to determine accuracy of different biomarkers as the diagnostic tests with the estimation of difference significance of the area under curves (AUC) larger than 0.5 in z-statistics. Logistic regression with stepwise option was chosen to find out which parameter was related to BHR.

A p-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using the MedCalc® version 10.0.1.0. software.

Results

During 3 months of phone recruitment to the study, 155 children were qualified and 149 of them were seen in the outpatient department. After subsequent questioning, 141 subjects fulfilled the inclusion criteria.

Correct spirometric tests were done by 139 children; in this group 110 presented with chronic rhinitis (61 with AR and 49 with NAR), 29 – with intermittent rhinitis (12 with AR and 17 with NAR).

Chronic rhinitis

There were no differences in the predicted values of FEV_1, FEV_1/FVC between children with AR and NAR in the group of chronic rhinitis. However, the values of $FEF_{25-75\%}$ were lower in the NAR group than in the AR group (difference of borderline significance (p = 0.084) (Table 1).

Bronchial hyperresponsiveness in the group with chronic rhinitis was seen in 12% (6/49) of subjects with NAR and in 28% (17/61) of subjects with AR (borderline significance in the difference; p = 0.077). The calculated odds ratio for BHR in AR in comparison to NAR was 2.768

Table 1. Spirometric parameters (% of predicted values, mean ± SD) in children with allergic rhinitis (AR) and nonallergic rhinitis (NAR) in chronic and intermittent rhinitis

	AR	NAR	Value of p
Chronic rhinitis	**n = 61**	**n = 49**	
FEV_1	100.8 ±12.9	102.7 ±11.1	0.433
$FEF_{25-75\%}$	106.0 ±25.4	98.2 ±20.5	0.084
FEV_1/FVC*	93.2 (91.2-94.4)	92.3 (87.2-95.0)	0.510
ΔFEV_1*	5.9 (4.8-8.0)	4.1 (2.5-5.7)	0.072
Intermittent rhinitis	**n = 12**	**n = 17**	
FEV_1	105.4 ±14.0	93.5 ±10.5	0.014
$FEF_{25-75\%}$	99.8 ±18.5	89.7 ±19.9	0.177
FEV_1/FVC*	91.0 (81.4-96.8)	90.2 (82.3-95.5)	0.642
ΔFEV_1*	2.9 (–0.6-11.4)	4.6 (0.6-11.9)	0.425

*ΔFEV_1 – relative drop of FEV_1 in the exercise challenge test, *median (95% confidence interval for median)*

with 95% CI (0.997-7.688) (p = 0.051). Moreover, the values of ΔFEV_1 in the NAR group were slightly lower as compared to these in the AR group (p = 0.072) (Table 1). Of note, in two subjects with AR and in one with NAR, the bronchodilator-response tests were done because their baseline FEV_1 values were less than 80% of the predicted values.

Intermittent rhinitis

Of all the analyzed spirometric parameters, only the mean value of FEV_1 was significantly lower in children with intermittent NAR in comparison to AR (p = 0.014) (Table 1). Bronchial hyperresponsiveness was observed in 5 children out of 17 (29%) with NAR and in two out of 12 (17%) with AR (p = 0.73). There were no significant differences in ΔFEV_1 in both groups (Table 1). In one child from the NAR group, the bronchodilator-response test was done (his baseline FEV_1 < 80% of the predicted value).

Table 2. Biomarkers of allergic inflammation (geometric mean and 95% CI) in children with allergic rhinitis (AR) and nonallergic rhinitis (NAR) in chronic and intermittent rhinitis

	AR	NAR	Value of p
Chronic rhinitis	**n = 61**	**n = 49**	
FeNO [ppb]	23.5 (19.3-28.7)	12.7 (10.9-14.7)	< 0.0001
tIgE [kU/l]	181.3 (125.8-261.3)	33.6 (23.9-47.3)	< 0.0001
IL-4 [pg/ml]	0.182 (0.177-0.187)	0.182 (0.173-0.192)	0.913
TNF-α [pg/ml]	1.134 (1.058-1.215)	1.277 (1.086-1.502)	0.113
Eos [cells/µl]*	238 (181.7-294.3)	126 (67.9-175.3)	0.002
Intermittent rhinitis	**n = 12**	**n = 17**	
FeNO [ppb]	23.3 (12.6-43.0)	11.7 (9.7-13.9)	0.034
tIgE [kU/l]	223.9 (114.4-438.6)	30.4 (10.4-88.3)	0.003
IL-4 [pg/ml]	0.18 (0.17-0.18)	0.17 (0.16-0.19)	0.859
TNF-α [pg/ml]	1.19 (1.04-1.35)	1.13 (0.83-1.55)	0.708
Eos [cells/µl]*	160 (56.5-307.3)	171 (39.0-273.8)	0.807

**Median (95% CI for median), 95% CI – 95% confidence interval*

Biomarkers of allergic inflammation

The characteristics and observed differences in the analyzed inflammatory biomarkers (tIgE, FeNO, IL-4, TNF-α, Eos) are presented in Table 2.

Accuracy of the allergic biomarkers in the diagnosis of AR in children with chronic or intermittent rhinitis

The parameters of the test accuracy for AR diagnosis with different biomarkers of allergic inflammations with the best cut-off level are displayed in Table 2. The results are shown together for chronic and intermittent rhinitis, because of a relatively small number of subjects in the intermittent group and no differences in diagnostic accuracy of the analyzed biomarker separately for chronic and intermittent rhinitis groups (results not shown).

The analysis of differences in AUC for tIgE and FeNO (AUC_{tIgE}-AUC_{FeNO} = 0.102; p = 0.042), FeNO and Eos (AUC_{FeNO}-AUC_{Eos} = 0.07; p = 0.219), tIgE and Eos (AUC_{tIgE}-AUC_{Eos} = 0.172; p = 0.002) demonstrated that the tIgE level was the test which in the best way differentiated between AR and NAR. The same conclusion can be drawn from the test accuracy parameters shown in Table 2 and from a graphical analysis of the position of appropriate ROC curves (Figure 1).

Relationship of allergic inflammation biomarkers with bronchial hyperresponsiveness

Logistic regression analysis did not include any analyzed biomarkers as a prediction factor of BHR in children with AR or NAR in the entire rhinitis group (chronic and intermittent).

Discussion

Despite the very similar prevalence of AR and NAR in children, the clinical characteristics and course of NAR are not fully understood [12]. Nowadays, the associations

Table 3. Tests accuracy for diagnosis of allergic rhinitis with different biomarkers of allergic inflammation

Biomarker	Cut-off level	Sensitivity	Specificity	(+)PV	(–)PV	AUC	95% CI for AUC	Value of p for AUC > 0.5
tIgE	68 kU/l	78.9	79.4	81.2	76.9	0.829	0.755-0.889	0.0001
FeNO	19.6 ppb	57.3	86.8	82.7	64.8	0.738	0.658-0.808	0.0001
Eos	150 cells/µl	70.3	60.9	67.5	63.9	0.661	0.576-0.740	0.0004

(+)PV – positive predictive value, (–)PV – negative predictive value, AUC – area under ROC curve, 95% CI – 95% confidence interval

between AR and other allergic diseases, especially asthma, are very well known and established. This close interrelationship between asthma and AR has been expressed in the concept of united airways for the several years [6, 13]. Much less is known about the effect of chronic inflammatory process on the respiratory system in NAR [1].

In this study, we aimed at characterizing the pulmonary function parameters in the group of children with NAR in relation to AR. Moreover, we tried to find the marker (except allergy testing) which would best differentiate these two rhinitic conditions. We believe that a positive side of this study is the way the children were recruited, which resembles the cohort of pediatric patients visiting outpatient departments because of chronic rhinitis.

The evaluation of spirometric parameters (FEV_1, $FEF_{25-75\%}$, FVC, FEV_1/FVC) did not demonstrate essential differences between groups of schoolchildren with NAR and AR. The observed significantly lower values of FEV_1 with intermittent rhinitis in NAR as compared to the AR group should be interpreted with caution because of a low number of analyzed subjects. In the present literature, there are no data available on the comparison of baseline spirometric parameters in children with AR and NAR. However, in adults and adolescents, Molgaard *et al.* did not show differences in spirometric values (FEV_1, FVC, FEV_1/FVC) in AR and NAR groups [14]. However, in their study, the authors indicated that nasal symptoms in NAR were more prominent and lasted longer than in AR. Besides, BHR in that study was less common in the NAR group [14].

The same situation was observed in the present study. The prevalence of bronchoconstrictor response after exercise was over 2.5 times higher (OR = 2.8) in AR than in NAR. On the other hand, a relative decrease of FEV_1 in the exercise challenge test in children was no different between the NAR and AR groups. A much greater difference in the prevalence of post exercise bronchoconstrictor response in children with chronic rhinitis was observed by Bronstein and Atlas. In their study, this response was noted in almost half of children (47.2%) with AR and 5% with NAR [15]. The difference between this study and Bronstein's investigations could be explained by changes in the epidemiological situation of chronic rhinitis (the study by Bronstein was done 20 years ago) and better standardization of the exercise challenge test at the present time [10]. Recently, in the published study in adults, metacholin-induced BHR was shown in 35% of subjects with AR and in 20% with NAR [14]. Another study indicated no difference in asthma and BHR prevalence in adults with AR and NAR [16]. Moreover, a large European study showed that chronic rhinitis (allergic and nonallergic) was an independent risk factor for BHR and asthma in adults [17]. It was demonstrated that there was a better relationship of asthma or rhinosinusitis with AR than with NAR [18] and the risk of asthma was almost 8 times higher in AR adult subjects in comparison to the normal population [19].

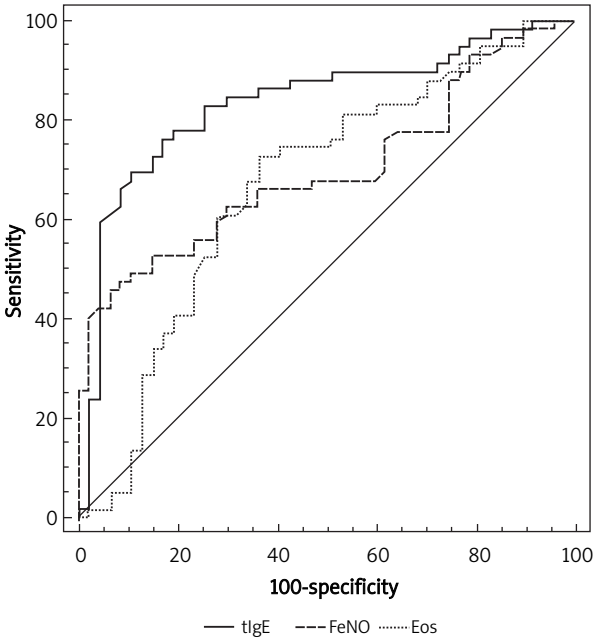

Fig. 1. ROC curve for comparison of diagnostic accuracy of allergic rhinitis with the different allergic inflammation markers in schoolchildren

Allergic rhinitis is characterized by polarization of immunological response towards Th2 lymphocytes. The involvement of cytokines, especially interleukin (IL)-4 and IL-5, was evaluated in the previous studies with the seasonal AR [20]. Interleukin-4 is a cytokine, which induces skewness into Th2 cells, stimulates the synthesis of IgE and adhesive molecules, which in turn increases recruitment of eosinophils into an allergic inflammation site and production of mucus. It could be suspected that this cytokine plays one of the main roles in propagation of the inflammatory process in AR [21]. The role of another cytokine – TNF-α – in the pathology of allergic inflammation is not completely determined. It is synthesized together with IL-1, IL-6, IL-8 by fibroblasts, endothelial and epithelial cells and macrophages under influence of IL-17 released by the specific subpopulation of lymphocytes [22]. Participation of TNF-α in maintaining allergic inflammatory process was demonstrated in several clinical studies [23-26].

Taking together the above data, in this study we compared the levels of IL-4 and TNF-α with other known allergy inflammation markers (e.g. FeNO, tIgE, eosinophilia level) in the AR and NAR groups of children. Moreover, we estimated the value of these markers in the differentiation of AR and NAR.

This study showed significantly higher levels of FeNO, tIgE and absolute eosinophils in chronic AR and FeNO, tIgE in intermittent AR in comparison to NAR. The difference in absolute levels of eosinophils was not more significant

in intermittent rhinitis. Moreover, the levels of cytokines IL-4 and TNF-α were not suitable for differentiation of children with AR or NAR. The differences in the levels of the determined biomarkers (FeNO, tIgE, absolute eosinophils) supported an analysis aiming at establishing which of them would be the best predictor for AR diagnosis. The comparison of ROC curves showed that measurement of tIgE was the test with the best accuracy. The 68 kU/l cut-off level gave approximately 80% of the sensitivity and specificity. In comparison, FeNO level of approximately 20 ppb gave sensitivity slightly above 60% and specificity of approximately 87%. None of the analyzed markers was significantly related to BHR in children with AR or NAR.

These results of our study are in concordance with studies in adults. Rolla *et al.* showed significantly higher FeNO levels in subjects with AR than in NAR; moreover, higher values were seen in these patients with asthma [18]. The evidence of the usefulness of FeNO measurement in AR was presented in the study by Henriksen *et al.* The level of this biomarker was higher during seasonal aeroallergens exposure [27]. However, there were no correlations between FeNO and parameters of pulmonary function tests in subjects with AR [28].

Moreover, there was no relationship between FeNO and BHR in children with asthma in other studies [29].

Rondon *et al.* demonstrated significant differences in tIgE levels between AR and NAR in adults, but without any differences between NAR and healthy subjects. In that study, serum ECP levels (analysis of eosinophils was not presented) were not different between the observed groups [30]. In the previous studies in children, differences were shown in peripheral eosinophils and ECP in serum related to diagnosis of AR or NAR, but only the ECP level correlated with nasal symptoms score [31]. In another study, significantly higher levels of tIgE, ECP and FeNO were observed in children with AR in relation to healthy children without atopy. The interesting finding of that study was that there was no effect of intranasal antiallergic treatment on FeNO levels with a concurrent decrease of ECP levels during this therapy [32].

In this study, we have demonstrated a relatively good discrimination value of serum tIgE levels for differentiation between AR and NAR and a moderate value for FeNO measurements. These observations are in agreement with the results of other authors, who established the usefulness of tIgE for the allergic diseases diagnosis. Moreover, a significant positive correlation between tIgE and FeNO in the group of children with AR was seen, being even better with the number of positive prick tests [33].

Conclusions

The comparison of groups of children with AR and NAR demonstrated no significant differences in their pulmonary function tests. The frequency of BHR estimated in the exercise challenge test was higher in the AR group than in the NAR group. None of the analyzed markers of allergic inflammation (FeNO, IL-4, TNF-α, tIgE, eosinophilia) was significantly related to BHR in children with rhinitis. However, the level 68 kU/l of tIgE was a marker, which with moderate accuracy diagnoses AR in schoolchildren.

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