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Elevated Serum Level of CD48 in Patients with Intermittent Allergic Rhinitis

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Keywords
CD48 · Allergic rhinitis · Intermittent allergic rhinitis · Nitric oxide · Fractional exhaled nitric oxide

Abstract
Background: In the pathogenesis of intermittent allergic rhinitis (IAR), the inflammatory reaction is of importance. CD48, belonging to the CD2 family, participates in mast cell-stimulating cross-talk, facilitates the formation of the mast cell/eosinophil effector unit, and is expressed by eosinophils. ***Objectives:*** To assess the serum level of soluble form of CD48 (sCD48) in patients with IAR during and out of the pollen season and correlate with the disease severity and with eosinophil-related parameters. ***Materials and Methods:*** Sixty-three patients (female: 79%; mean age: 30.58) were included to the study. Forty-five patients were assessed during the pollen season and other 42 patients during out of the pollen season. Twenty-four patients (female: 37.50%; mean age: 27.90) were evaluated twice, during the pollen season and out of the pollen season. sCD48, ECP, eotaxin-1/CCL11 serum levels together with complete blood count, and fractional exhaled nitric oxide bronchial and nasal fraction (FeNO) were performed. The severity of symptoms was assessed using the Total Nasal Symptom Score (TNSS), and neutrophil-to-lymphocyte (NLR) and eosinophil-to-lymphocyte (ELR) ratios were calculated. ***Results:*** sCD48 serum level, FeNO nasal and bronchial fractions, and TNSS were significantly higher in the IAR group in the pollen season compared with out of the pollen season. Differences in ECP, eotaxin-1/CCL11 serum levels, and NLR and ELR were not significant between season and out of the season. No correlations were found between sCD48 and eosinophil-related parameters. ***Conclusions:*** sCD48 may be a biomarker to the exacerbation phase in patients with IAR. One can assume that CD48 participates in the pathogenesis of IAR.

Introduction

Allergic diseases are currently one of the main medical, social, and economic problems. The World Health Organization (WHO) has estimated that 400 million people in the world suffer from allergic rhinitis (AR). In Europe, the European Community Respiratory Health Survey established the prevalence of AR as being from 4 to 32% [1, 2]. AR is clinically defined as a symptomatic disorder of the nasal mucosa induced by allergen exposure and mediated by IgE [3, 4]. Intermittent AR (IAR) based

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on Allergic Rhinitis and its Impact on Asthma (ARIA) is characterized with symptoms lasting for <4 days/week or <4 consecutive weeks [4–6]. The most common symptoms include repeated sneezing, itching, blocked nose, and watery nasal discharge. In addition, more complex symptoms such as sleep disorders, problems with concentration, and general fatigue may appear [3–6]. AR significantly reduces comfort and quality of life and is also a frequent cause of visits to a general practitioner.

CD48 is a glycosylphosphatidylinositol protein present as a membrane receptor (mCD48) on hematopoietic cells and as a soluble form (sCD48) in serum [7]. CD48 can act as an adhesion protein, and it also has a costimulatory molecule on lymphocytes [7]. CD48 exists on the surface of a few hematopoietic cells such as monocytes, neutrophils, mast cells, and eosinophils as well as B and T lymphocytes, natural killer cells, and dendritic cells [8]. CD48 contributes to several immunological processes through its interactions with ligands CD2 and CD244. CD48 expression increases under inflammatory conditions. Exposure to cytokines such as IFNα, IFNβ, and IFNγ can increase CD48 expression on human peripheral blood mononuclear cells [9]. Patients with EBV infection or arthritis exhibit elevated levels of sCD48 in the serum [10]. CD48 increased in *Staphylococcus aureus* infections in atopic dermatitis [11]; also, staphylococcal enterotoxin B (SEB) regulates CD48 dynamics and formation of sCD48 from eosinophils in vitro and in vivo [12]. During allergic responses, CD48 expression increases on eosinophils [13, 14]. It has been discovered that expression of CD48 on eosinophils is regulated by IL-3 mediated by orosomucoid 3 [14, 15]. The CD48 antigen has a high affinity to a 2B4 ligand found on the surface of eosinophils, thereby forming an "allergic effector unit" [16]. Stimulation of CD48 on the surface of eosinophils by binding to specific antibodies leads to the release of eosinophilic proteins: eosinophilic peroxidase and eosinophilic neurotoxin (eosinophil-derived neurotoxin) [14]. The observed relationships may be important in the pathogenesis of AR.

The importance of CD48 in the pathogenesis of allergic diseases has been proven in asthma. A significant increase in the serum concentration of sCD48 was observed in patients with chronic mild asthma and chronic moderate asthma. sCD48 values allowed to distinguish between mild and moderate asthma from the healthy group. Increased serum concentrations of sCD48 have also been demonstrated in patients with nonallergic asthma compared with allergic asthma and healthy people [17–20]. The importance of CD48 in other allergic diseases, including AR, has not yet been studied.

In the present study, we aimed to assess the serum level of sCD48 in patients with IAR during and out of the pollen season and to correlate with the disease severity. An additional aim was to analyze if there is a correlation between the serum level of sCD48 and the number of peripheral blood eosinophils, eosinophil-to-lymphocyte ratio (ELR), neutrophil-to-lymphocyte ratio (NLR), 2 other proteins related to the functioning of eosinophils – eosinophil cationic protein (ECP) and chemokine eotaxin-1/CCL11 – and the amount of nitric oxide (FeNO) in the bronchial and nasal fraction of exhaled air. Confirmation of the role of CD48 in IAR may have clinical relevance as a new molecular pharmacological target.

Methods

From January 2018 to October 2019, volunteer subjects were diagnosed at the Department of Internal Disease, Allergology and Clinical Immunology in University Clinical Hospital named K. Gibińskiego Silesian Medical University in Katowice, Poland. The patients were examined on 2 occasions: during the period of exacerbation in allergen season (spring and summer) and during remission, that is, out of season (autumn and winter). The research was approved by the Bioethics Committee at the Silesian Medical University in Katowice, Resolution No. KNW/0022/KB1/66/1/12/18. All subjects were informed about planned procedures, and written informed consent was obtained from all patients.

Inclusion criteria were as follows: patients of both genders, ranging in age from 18 to 70 years, diagnosis of IAR based on ARIA guidelines [3], no pharmacological treatment at the time of inclusion to the study, and informed written consent. Exclusion criteria were as follows: acute and chronic infections, other inflammatory conditions of the respiratory system (including asthma), other acute and chronic diseases (e.g., cardiac, renal, and gastrointestinal disease) which in the researcher's opinion may affect the results obtained, nasal polyposis, use of antihistamines for at least 7 days prior to the study, long-acting systemic glucocorticosteroids for 3 weeks, short-acting systemic glucocorticosteroids (<50 mg) for 3 days, topical glucocorticosteroids for 2 weeks prior to the study including intranasal glucocorticosteroids 2 days before examination, AR without symptoms over last 2 years, lack of a patient consent, and pregnancy.

Research Plan

Patients with IAR were evaluated in a symptomatic phase of disease, during natural exposure to aeroallergens such as tree pollen (from February to April) and grass pollen (from May to July), or in an asymptomatic period in autumn and winter. A part of the patients from the study group (IAR twice group) were evaluated twice, both in and out of the pollen season. In each patient, a detailed interview was conducted, skin prick tests (SPT) were performed, and IAR diagnosis was made based on the concordance between the reported symptoms and SPT results. At each assessment, the evaluation of severity of nasal symptoms with use of Total Nasal Symptom Score (TNSS) questionnaire and determina-

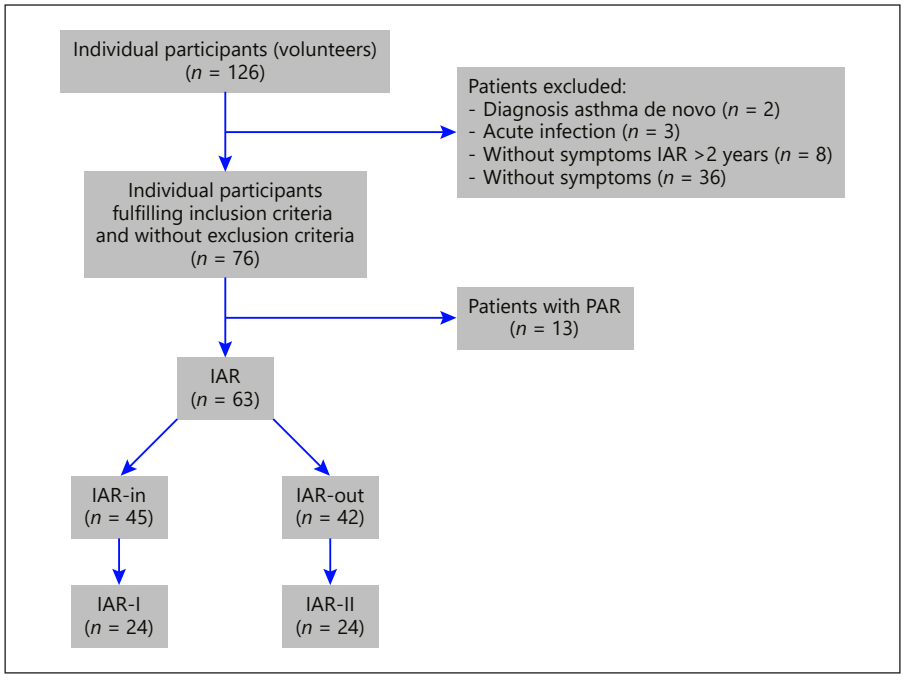

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Fig. 1. Flowchart of qualification to the study.

tion of FeNO concentration in the nasal and bronchial fraction were performed, and the blood sample was collected to determine sCD48, ECP, and eotaxin-1/CCL11 serum levels together with complete blood count. Complete blood count estimation was performed in 3 or 4 h after venopuncture, and the serum sample was properly secured and stored in the freezer at −80°C for further laboratory estimations.

Patient Evaluation

Patient assessments including demographic data such as sex, age, and clinical data, medical interview, time since diagnosis, comorbidities, treatment of IAR, previous immunotherapy, duration of IAR (days with symptoms per week and number of consecutive weeks with symptoms according to ARIA) [3], and IAR severity (TNSS), were performed as inclusion into the study.

Skin Prick Tests

Skin prick tests were performed with the following allergens: grass pollen, cereals, rye, tree (birch, alder, hazel, and beech) pollen, mugwort, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, *Penicillium notatum*, *Aspergillus fumigatus*, and animal dander on the forearm. Histamine (10 mg/mL) and physiological saline (0.9% NaCl) were taken as positive and negative controls. Skin reaction was evaluated after 15 min. The positive results were taken as an average wheal diameter >3 mm and greater than the negative control.

Severity Evaluation

AR symptoms were assessed using the TNSS by calculating the sum of scores for nasal congestion, rhinorrhea, nasal itching, sneezing, and difficult sleep. Symptoms were rated on a scale of 0–3, with 0 meaning no symptoms and the 3 meaning most pronounced symptoms in the last 2 weeks. The maximum number of points was 15. The severity was assessed as mild for points 0–5; moderate, 6–10; and severe, 11–15.

Table 1. Characteristics of patients

	IAR (*N*)	IAR-in	IAR-out	IAR-I and IAR-II
N (%)	63	45 (71.42)	42 (66.66)	24 (38.01)
Age, years	30.58	29.52	32.71	27.90
Male, *n* (%)	31 (49)	24 (53)	21 (50)	15 (62.50)
Female, *n* (%)	32 (51)	21 (47)	21 (50)	9 (37.50)

IAR, intermittent allergic rhinitis.

Determination of FeNO Concentration in the Nasal and Bronchial Fraction of Exhaled Air

The test was performed using the HypAir FeNO device from Medisoft. Bronchial fraction measurements were performed through a disposable head with an antibacterial filter, using a single-breath technique in which the patient inhaled nitric oxide-free air to the level of maximum inspiration (total lung capacity) and then exhaled at 50 mL/s (under control of resistance device and patient feedback displayed on the monitor) for about 6 s. The nasal fraction consisted of making calm breaths and exhalations into the adapter connected to the nasal cavity in a specific unit of time – 20 s.

Laboratory Estimations

The antecubital vein was used to gain samples of 5 mL for ELISA and 5 mL for total blood count following 12 h of fasting. The

Table 2. Assessment of sCD48, ECP, eotaxin-1/CCL11 level, FeNO nasal and bronchial fraction, ELR, NLR, and TNNS values in the group of patients with IAR who were assessed twice – during the pollen season (IAR–I) and out of the pollen season (IAR-II)

	IAR–I (n = 24)	IAR-II (n = 24)	p value*
sCD48, pg/mL	7,483 (5,206.0–12,010.0)	6,181.5 (4,999.0–8,934.0)	0.00005
Eotaxin-1/CCL11, pg/mL	34.5 (15.0–79.0)	34 (14.0–51.0)	ns
ECP, pg/mL	78.5 (50.0–393.0)	81.0 (60.0–632.0)	ns
FeNO nasal fraction, ppb	n = 20 2,022.5 (378.0–2,260.0	n = 20 1,297.0 (234.0–2,174.0)	0.003
FeNO bronchial fraction, ppb	18.5 (6.0–57.0)	16.0 (5.0–46.0)	0.027
Eosinophils, 10^3/μL	n = 18 0.26 (0.05–0.62)	n = 18 0.22 (0.06–0.76)	ns
ELR	16.33 (4.74–101.80)	16.25 (0–97.86)	ns
NLR	1.34 (0.82–15.52)	1.52 (0.68–3.55)	ns
TNSS (2 weeks)	5.0 (1.0–15.0)	0 (0.0–5.0)	0.00003

IAR, intermittent allergic rhinitis; ECP, eosinophil cationic protein; FeNO, fraction exhaled nitric oxide; ELR, eosinophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; TNSS, Total Nasal Symptom Score; ns, nonsignificant. * Wilcoxon test.

blood samples were collected in a hematologic sample tube containing an anticoagulant, and complete blood counts were recorded using a Sysmex XN-350 (Sysmex Europe Corp, Norderstedt, Germany) hematology analyzer. Using these data, neutrophil and eosinophil absolute numbers were divided by the lymphocyte absolute number, and NLR and ELR values were calculated.

The serum level of sCD48 was evaluated using the sCD48 ELISA kit (Catalog No. SK00494-01; Aviscera Bioscience, Inc., Santa Clara, CA, USA); ECP concentration – Ribonuclease A3 (RNASE3) ELISA (No. SEB758Hu; Cloud-Clone Corp., Houston, TX, USA); and eotaxin concentration – Eosinophil chemotactic factor (ECF) ELISA (No. SEA025Hu; Cloud-Clone Corp., Houston, TX, USA). Sensitivity of sCD48 determinations is <7.5 pg/mL, ECP 34 pg/mL, and eotaxin 5.7 pg/mL.

Statistical Analysis

Data are presented as median with interquartile range for variables with nonnormal distribution and mean ± SD for variables with normal distribution. To evaluate normality of variables, the Shapiro-Wilk test was used. The Mann-Whitney U test to compare nonnormal variables, the Kruskal-Wallis test for >2 groups, the Wilcoxon test for dependable values, and Spearman's rank test were used to evaluate associations between variables. p values <0.05 were considered significant. Analysis was performed using STATISTICA 13.3 software (StatSoft, Poland).

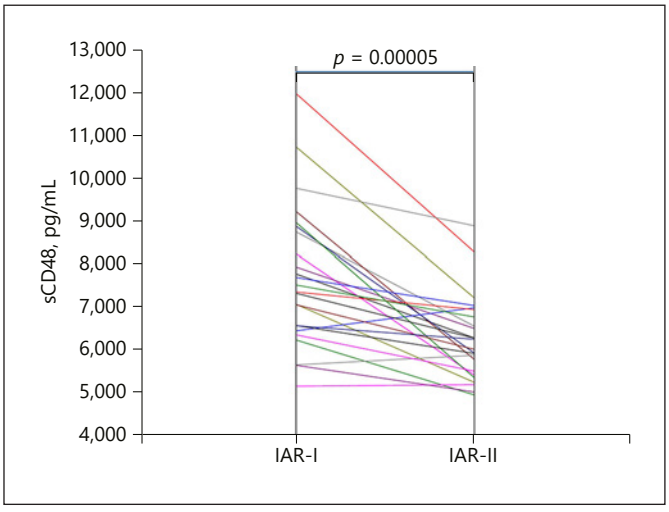

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Fig. 2. Serum levels of sCD48 in the IAR-I group during the pollen season and IAR-II group out of the pollen season period (Wilcoxon test). sCD48, soluble form of CD48; IAR, intermittent allergic rhinitis.

Results

126 individuals with different symptoms of chronic rhinitis were analyzed, and eventually 63 patients (50 female [79%] at a mean age of 30.58; range 18–60 years) with no exclusion criteria were included to the final analysis. Forty-five patients (71.42%; female: 46.6%; mean age: 29.52 years) were assessed during the pollen season period (group IAR-in) and other 42 patients (66.66%; female: 50%; mean age: 32.71 years) during out of the pollen season (group IAR-out). Twenty-four patients (38.01%; female: 37.50%; mean age: 27.90 years) were evaluated twice, during the symptomatic period of pollen season (group IAR-I) and out of the pollen season (group IAR-

Table 3. Assessment of sCD48, ECP, eotaxin-1/CCL11, FeNO nasal and bronchial fraction, peripheral blood count, NLR and ELR, and TNSS values for the study group with IAR during pollen season and out of pollen season period (IAR-in and IAR-out)

	IAR-in (n = 44)	IAR-out (n = 42)	p value*
sCD48, pg/mL	6,783.5 (4,604.0–12,010.0)	6,063.0 (1,006.0–9,224.0)	0.005
Eotaxin-1/CCL11, pg/mL	39.5 (15.0–97.0)	37.0 (14.0–80.0)	ns
ECP, pg/mL	80 (50.0–393.0)	81.0 (53.0–632.0)	ns
FeNO nasal fraction, ppb	n = 37 2,022.0 (378.0–2,260.0)	n = 38 1,213.5 (196.0–2,174.0)	0.0001
FeNO bronchial fraction, ppb	19.0 (5.0–76.0)	13.0 (5.0–92.0)	0.02
Eosinophils, 10^3/μL	n = 41 0.24 (0.05–0.62)	n = 38 0.17 (0.04–0.76)	0.04
Leukocyte count, 10^3/μL	6.33 (3.7–12.4)	6.41 (3.70–11.70)	ns
Neutrophil count, 10^3/μL	3.41 (1.4–45.0)	3.55 (2.03–8.77)	ns
Lymphocyte count, 10^3/μL	2.10 (0.61–3.78)	2.09 (0.61–4.00)	ns
ELR	16.33 (4.74–101.80)	18.75 (0–111.20)	ns
NLR	1.56 (0.82–15.51)	1.54 (0.56–7.26)	ns
TNSS (2 weeks)	n = 44 5.5 (4.0–8.0)	n = 42 0 (0.0–3.0)	0.0000

IAR, intermittent allergic rhinitis; ECP, eosinophil cationic protein; ELR, eosinophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; FeNO, fraction exhaled nitric oxide; TNSS, Total Nasal Symptom Score; ns, nonsignificant. * Mann-Whitney U test.

II), shown in Fig. 1. The groups were comparable in terms of age and sex distribution ($p > 0.05$), shown in Table 1. The sCD48 serum level was significantly higher both in the whole IAR group estimated during season (IAR-in) as compared with IAR-out ($p < 0.005$; Mann-Whitney U test) and in the group of patients from the group IAR-I as compared with the group IAR-II ($p < 0.00005$; Wilcoxon test) (shown in Tables 2, 3; Fig. 2, 3).

In 24 (53%) patients in the IAR-in group, the TNSS after 2 weeks was of mild degree (0–5 points), in 17 (38%) patients, moderate (6–10 points) and in 4 (9%) patients, severe (11–15 points). In the IAR-out group, mild degree was found in 38 (91%) patients and most of them had only 0 or 1 point, moderate in 3 (7%) patients, and severe in only 1 (2%) patient.

FeNO nasal and bronchial fraction, eosinophils, and TNSS after 2 weeks were significantly higher in the IAR-in group compared with the IAR-out group ($p < 0.0001$; $p < 0.02$; $p < 0.04$; $p < 0.0000$, respectively; Mann-Whitney U test) (shown in Table 3; Fig. 4). FeNO nasal and bronchial fraction and TNSS after 2 weeks were significantly higher in the IAR-I group compared with the IAR-II group ($p < 0.03$; $p < 0.027$; $p < 0.00003$, respectively; Wilcoxon test) (shown in Table 2; Fig. 5).

In 13 (54%) patients in the IAR-in group, the TNSS after 2 weeks was of mild degree (0–5 points), in 10 (42%) patients, moderate (6–10 points) and in 1 (4%) patient, severe (11–15 points). In the IAR-out group, mild degree was found in 24 (100%) patients and most of them had 0 point, moderate 0 and severe 0. No differences in ECP, eotaxin-1/CCL11 serum levels, and NLR and ELR were

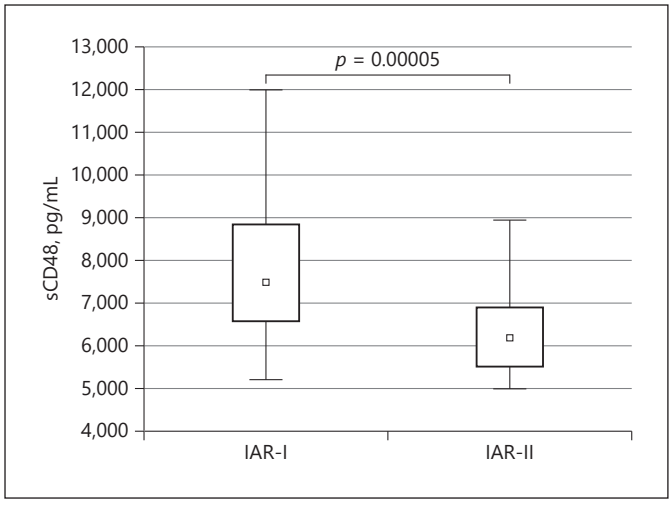

Fig. 3. Serum levels of sCD48 in the IAR group during the pollen season and out of the pollen season period (Wilcoxon test). sCD48, soluble form of CD48; IAR, intermittent allergic rhinitis.

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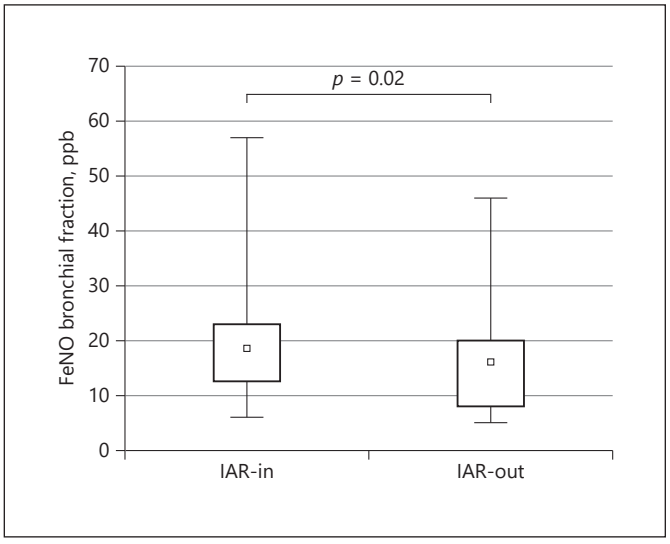

Fig. 4. Concentrations of fraction exhaled nitric oxide bronchial fraction in the IAR group during the pollen season and out of the pollen season period (Mann-Whitney U test). IAR, intermittent allergic rhinitis; FeNO, fraction exhaled nitric oxide.

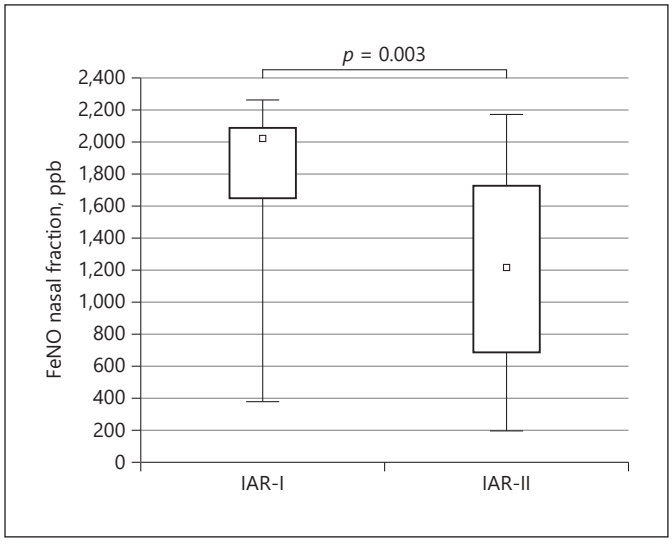

Fig. 5. Concentrations of fraction exhaled nitric oxide nasal fraction in the IAR group during the pollen season and out of the pollen season period (Wilcoxon test). IAR, intermittent allergic rhinitis; FeNO, fraction exhaled nitric oxide

Table 4. Evaluation of TNSS in patients in the IAR-in group (during pollen season)

TNSS	IAR-in			
	mild, $n = 24$	moderate, $n = 16$	severe, $n = 4$	p value*
sCD48, pg/mL	6,670.5 (5,206.0–10,766)	7,108.5 (4,604.0–12,010.0)	6,144.50 (6,045.0–8,919.0)	ns
Eotaxin-1/CCL11, pg/mL	43.5 (16.0–97.0)	28.5 (15.0–64.0)	36.50 (30.0–43.0)	0.02
ECP, pg/mL	84.0 (56.0–393.0)	76.5 (50.0–288.0)	106.5 (70.0–349.0)	ns
FeNO nasal fraction, ppb	$n = 17$ 2,023 (1,088.0–2,239.0)	1,913.5 (504.0–2,260.0)	1,174 (378.0–2,089.0)	ns
FeNO bronchial fraction, ppb	21 (6.0–76.0)	17.0 (6.0–50.0)	24.5 (5.0–57.0)	ns
Eosinophils, $10^3/\mu L$	$n = 20$ 0.19 (0.06–0.62)	0.30 (0.05–0.50)	0.22 (0.11–0.31)	ns
ELR	17.41 (4.74–60.57)	13.80 (5.23–101.80)	13.30 (7.77–24.81)	ns
NLR	1.41 (0.82–3.50)	1.93 (0.82–15.52)	1.60 (1.25–2.03)	ns

IAR, intermittent allergic rhinitis; TNSS, Total Nasal Symptom Score; ECP, eosinophil cationic protein; ELR, eosinophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; FeNO, fraction exhaled nitric oxide; ns, nonsignificant. * Kruskal-Wallis test.

observed neither in IAR-in and IAR-out groups nor in IAR-I and IAR-II groups (shown in Tables 2, 3). No correlations were found between serum level of CD48 and ECP, eotaxin-1/CCL11, FeNO nasal or bronchial fraction, eosinophils, and NLR or ELR in IAR patients neither during the pollen season nor out of the pollen season (data not shown). A statistically significant relationship between serum eotaxin-1/CCL11 levels and the severity of symptoms (TNSS) in the IAR pollen season period group was demonstrated ($p < 0.02$) (shown in Table 4; Fig. 6).

Discussion

In the present study, we assessed serum level of sCD48 in patients with IAR in the symptomatic phase (pollen season) and asymptomatic phase (out of the pollen sea-

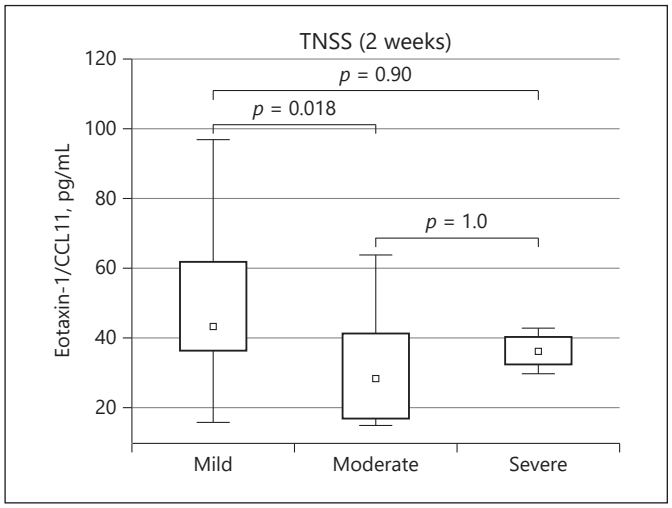

Fig. 6. Serum level of eotaxin-1/CCL11 in patients in the IAR-in group divided into 3 subgroups according to the TNSS (Kruskal-Wallis test). IAR, intermittent allergic rhinitis; TNSS, Total Nasal Symptom Score.

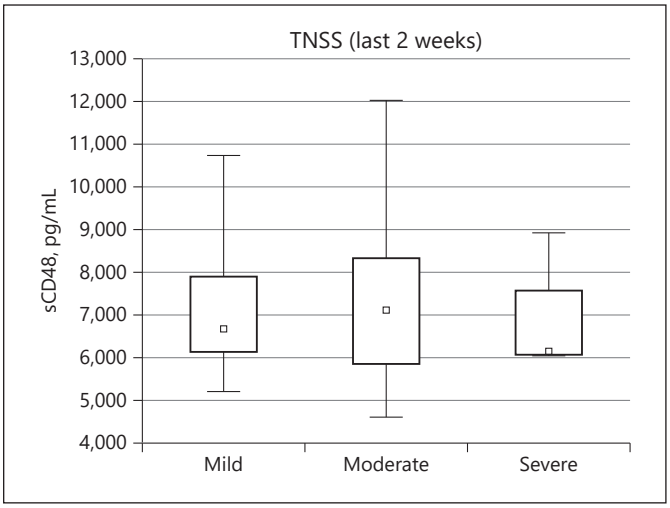

Fig. 7. Serum level of sCD48 in patients in the IAR-in group divided into 3 subgroups according to the TNSS (Kruskal-Wallis test; $p > 0.05$). IAR, intermittent allergic rhinitis; TNSS, Total Nasal Symptom Score.

son). We compared sCD48 findings with serum levels of ECP and eotaxin-1/CCL11, peripheral blood eosinophil number, ELR and NLR, and FeNO nasal and bronchial fraction results.

We found that the serum level of sCD48 may be a marker of allergic diseases, such as IAR. Furthermore, sCD48 is a marker of the IAR exacerbation as it significantly increased during the natural exposure to grass and tree pollen allergens as compared with out of the season. These findings suggest that sCD48 may play a role in IAR and may be useful as a laboratory marker. However, we failed to show that the sCD48 correlated with other proteins related to the functioning of eosinophils – ECP and eotaxin-1/CCL11. sCD48 did not correlate with peripheral blood eosinophil number and ELR and NLR; also, it was independent to the severity of IAR assessed by the TNSS.

Based on the TNSS questionnaire, we observed higher serum levels of sCD48 in patients with moderate intensity of IAR as compared with mild degree and lower values in patients with high intensity of symptoms. However, these differences were not statistically significant. It should be underlined that there were only 4 patients with severe degree, that is, points >10 in the studied group (shown in Fig. 7).

The role of CD48 as a costimulatory receptor of various immune responses is still being studied. The previous papers concentrated mainly on autoimmune diseases such as systemic sclerosis [18] or systemic lupus erythematosus [19], cancer (multiple myeloma [19] and acute myeloid leukemia [20]), and bacterial and other infectious diseases [21]. Interactions between CD48 and CD244 (2B4) on mast cells, eosinophils, and basophils suggest that these cell types can act synergistically in the "allergic effector unit" to promote inflammation. The 2B4-CD48 axis appears as a mechanism by which mast cells and eosinophils bind and stimulate one another in allergy. Therefore, we think that sCD48 may also be relevant in IAR pathophysiology. The role of sCD48 has been shown in allergic diseases mainly in bronchial asthma and indirectly in atopic dermatitis. Gangwar et al. [17] showed that membrane and soluble forms of CD48 were elevated in moderate asthma and they were downregulated in severe asthma. Increased serum levels of sCD48 have been demonstrated in patients with nonallergic asthma compared with allergic and healthy asthma as well [17]. This is important because AR is very often associated with asthma, and this result is in concordance with our findings of increased serum levels of sCD48 in nontreated patients with IAR in the pollen season. Munitz et al. [14] demonstrated that expression of CD48 on eosinophils from asthmatics was regulated by IL-3, and in experimental murine model of asthma, upregulation of CD48 in the OVA-challenged lungs was observed [13]. Interestingly, recently it has been shown that anti-CD48 mAb treatment significantly reduced airway inflamma-

tion in an allergy model that strongly suggests that this molecule may also have clinical relevance [13].

Minai-Fleminger et al. [11] studied the effect of *Staphylococcus aureus*, which is known to cause colonization of skin in patients with atopic dermatitis. They observed a significant increase in CD48 expression in the skin and on eosinophils of patients with atopic dermatitis, especially on the infiltrated eosinophils compared with normal nonatopic individuals. CD48 expression in vitro was also upregulated on human peripheral blood isolated eosinophils in the presence of *Staphylococcus aureus* exotoxins (SEB, protein A, and PGT) [11]. A later study showed that SEB regulates CD48 dynamics and formation of sCD48 from eosinophils and correlated with the activation state of eosinophils [12]. Taking together the results of our study and previously published papers confirms the role of sCD48 in allergic diseases, and among them IAR.

Apart from our results concerning sCD48, some other interesting findings were found in our study. First, we observed significantly increased FeNO nasal fraction in patients with IAR during the pollen season compared with out of the pollen season. Similar observations have been made earlier in patients with PAR. Significantly higher nasal fraction of FeNO was observed in patients with AR and/or without asthma [22] compared with the control subjects [23]. Liu et al. [24] also observed significantly higher FeNO nasal fraction values in patients with AR compared with the control group. The aforementioned research and our observations show that FeNO can be used as a quick and simple test for the differentiation of rhinitis. The latest European Position Paper on Rhinosinusitis and Nasal Polyps guidelines confirm that higher FeNO nasal fraction values are observed in patients with AR than those with other types of rhinitis [25]. Also, we noticed similar observation in FeNO bronchial fraction. Patients with IAR had statistically significantly increased FeNO bronchial fraction during the symptomatic period compared with the asymptomatic phase. So far, such observations have not been performed in such selective group of patients with IAR in exacerbation or asymptotic phases. It is in line with earlier studies which demonstrate that FeNO measurement is a method of evaluation of lower airway inflammation and may be employed for predicting bronchial hyperresponsiveness in patients with AR and its potential progression to asthma [26]. FeNO measurements might be used to improve diagnosis and intensity of IAR. Unfortunately, in our study we did not confirm the correlation between sCD48 and FeNO; thus, we may suppose that sCD48 is involved in different pathways in IAR development than nitric oxide.

In addition, we calculated the ELR and NLR in the IAR because so far they have not been counted in such a selective group and in 2 different periods as pollen season and out of the pollen season. In our study, we did not observe significant changes in NLR or ELR in patients with IAR in the pollen season as compared with cases out of the pollen season. Previously, a study of Göker et al. [27] confirmed significance of NLR in patients with PAR and Yalidz et al. [28] confirmed significance of ELR in patients with AR without dividing them into intermittent or perennial as compared with the control nonallergic group. Our findings may be explained by the fact that intensity of systemic inflammation is much lower in IAR than in other allergic diseases, such as PAR.

We also examined the serum level of chemokine eotaxin-1/CCL11, which is the chemotactic factor responsible for the migration of eosinophils to the site of allergic inflammation [29] and compared it with serum sCD48 levels. We did not find a correlation between eotaxin-1/CCL11 and sCD48 in the IAR. However, we observed the changing behavior of serum eotaxin-1/CCL11 level that was dependent on TNSS, but interestingly the highest levels were found in patients with moderate intensity of symptoms. This finding needs further research as it was not the main aim of our study.

We also assessed the serum levels of ECP which is released after degranulation of eosinophils and associated with an allergic process. The previous studies on AR showed increased serum levels of ECP in the pollen period as compared with remission [30], which has also been confirmed in our study, however, without statistical significance as serum level of ECP did not correlate with sCD48 in our study. Finally, we found that sCD48 is independent of other indicators of eosinophil activity.

Earlier studies confirming the role of CD48 in different types of asthma and our data suggesting the role of CD48 in IAR may indicate the direction for future research aimed at blocking of CD48 as a therapeutic option. The first data on anti-CD48 therapeutic effect murine model of asthma have been published [13].

A limitation of the present study is a relatively small number of participants in the groups and the fact that not all patients were estimated twice both in and out of the pollen season. A part of participants did not come again for follow-up because of different reasons. Moreover, restrictive exclusion criteria concerning the therapeutic requirements reduced the number of patients eligible for inclusion. Another limitation is a lack of healthy nonallergic control group. However in the project of our study,

direct comparisons between patients with IAR in and out of the season were planned so the estimations of results out of the season were used as a control.

Conclusion

sCD48 may have a role in the pathogenesis of IAR. Nasal fraction of FeNO may be a useful marker of IAR. Increased content of FeNO in the bronchial fraction in exhaled air may indicate systemic changes occurring in IAR. A better understanding of the roles of CD48 and its ligands on specific cell types will enable us to translate this knowledge to treatment to allergy.

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Statement of Ethics

This research was approved by the Bioethics Committee at the Silesian Medical University in Katowice, Resolution No. KNW/0022/KB1/66/1/12/18.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Olga Branicka: designed the study, analyzed and interpreted the data for the work, made tables and graphs, wrote the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Barbara Rogala: substantially contributed to the design of the work, revised the manuscript critically for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Edyta Jura-Szołtys: participated in revising article critically for the important intellectual content and final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Joanna Glück: substantially helped in designing the study, contributed to the conception and design of the work, interpretation of data for the work, and revising the work critically for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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