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Evaluation of selected biomarkers in the diagnosis of asthma in children

Ocena wybranych biomarkerów w diagnostyce astmy u dzieci

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Abstract

Introduction and objective: Asthma is one of the most common chronic diseases in the paediatric population. The aim of this study was to evaluate selected biomarkers: fractional exhaled nitric oxide (FeNO), eosinophil count, periostin; and potential biomarkers: progranulin, matrix metalloproteinase-9 (MMP-9) and tumour necrosis factor alpha (TNFα) in the diagnosis of asthma in children. **Materials and methods:** The study group consisted of 25 children with atopic asthma in a stable period of the disease, aged 6–17 years. The control group consisted of 21 healthy children aged 6–18 years with no history of allergic diseases. **Results:** Significantly higher serum periostin levels were observed in children with asthma compared to the control group. There were no statistically significant differences in progranulin, periostin and MMP-9 levels between asthmatic children vs. healthy controls, or between patients with controlled vs. uncontrolled asthma. Children with uncontrolled asthma had significantly higher FeNO levels compared to children with controlled asthma. Exhaled FeNO levels were significantly higher in children with eosinophil levels above $0.3 \times 10^3/\mu L$ than in those with eosinophil levels below $0.3 \times 10^3/\mu L$. Children with elevated FeNO had higher progranulin levels compared to children with normal FeNO values. There was a statistically significant positive correlation between FeNO and serum progranulin levels. **Conclusions:** Periostin may be used as a biomarker of atopic asthma in children. The applicability of FeNO and eosinophil count in the diagnosis of asthma in children has been confirmed. The role of progranulin and MMP-9 in the pathogenesis of asthma and their usefulness as a biomarker in children requires further study.

Keywords: asthma, biomarkers, periostin, progranulin, FeNO

Streszczenie

Wprowadzenie i cel: Astma jest jedną z najczęstszych chorób przewlekłych w populacji dziecięcej. Celem pracy była ocena wybranych biomarkerów: periostyny, stężenia tlenku azotu w wydychanym powietrzu (*fractional exhaled nitric oxide*, FeNO), liczby eozynofilów i potencjalnych biomarkerów: progranuliny, metaloproteinazy macierzy-9 (*matrix metalloproteinase-9*, MMP-9) i czynnika martwicy nowotworu alfa (*tumour necrosis factor alpha*, TNFα) w diagnostyce astmy u dzieci. **Materiał i metody:** Grupę badaną stanowiło 25 dzieci w wieku 6–17 lat z astmą alergiczną w stabilnym okresie choroby. Do grupy kontrolnej zakwalifikowano 21 zdrowych dzieci w wieku 6–18 lat, bez chorób alergicznych w wywiadzie. **Wyniki:** U dzieci z astmą zaobserwowano istotnie wyższe stężenie periostyny w surowicy w porównaniu z grupą kontrolną. Nie wykazano istotnych statystycznie różnic w zakresie stężeń progranuliny i MMP-9 pomiędzy dziećmi z astmą a zdrowymi, a także wśród chorych z astmą kontrolowaną i niekontrolowaną. W grupie dzieci z astmą niekontrolowaną wartości FeNO były istotnie statystycznie wyższe niż u dzieci z astmą kontrolowaną. W grupie badanej wśród dzieci z liczbą eozynofilów $>0,3 \times 10^3/\mu l$ wykazano istotnie statystycznie wyższe wartości FeNO w wydychanym powietrzu niż w grupie dzieci z liczbą eozynofilów $<0,3 \times 10^3/\mu l$. U dzieci z astmą i podwyższonymi wartościami FeNO stwierdzono istotnie statystycznie wyższe stężenie progranuliny w surowicy. Wykazano istotną statystycznie dodatnią korelację pomiędzy wartościami FeNO a stężeniem progranuliny w surowicy. **Wnioski:** Periostyna może służyć jako biomarker astmy alergicznej u dzieci. Potwierdzono przydatność FeNO i liczby eozynofilów w diagnostyce astmy alergicznej w populacji pediatrycznej. Ustalenie roli progranuliny i MMP-9 w patogenezie astmy i ich użyteczności jako biomarkerów u dzieci wymaga dalszych badań.

Słowa kluczowe: astma, biomarkery, periostyna, progranulina, FeNO

INTRODUCTION

According to the Global Initiative for Asthma (GINA) 2023 Report, asthma is a heterogeneous chronic inflammatory airway disease, defined by the history of respiratory symptoms, which may fluctuate over time and intensity, accompanied by varying limitations of expiratory airflow[1].

Asthma is one of the most common chronic diseases affecting both children and adults. A significant increase in its prevalence has been found over the past few decades. As reported by the World Health Organization (WHO), asthma affected an estimated 262 million people in 2019 and caused 461,000 deaths[2,3]. According to data from the National Health Fund, there were 2.2 million (5.7% of the population) people living with asthma in Poland in 2019, and this number remained at a similar level as in 2013–2019. In terms of population, the highest number of patients was observed in the age group of 6–10 years, particularly in boys (15,200 per 100,000 children in this age group)[4].

Asthma onset can occur at any age. In the case of allergic asthma, medical history usually confirms symptom onset in early childhood. According to epidemiological studies, 40% of children with first symptoms before the age of 6 years will development asthma. It is therefore crucial to diagnose asthma in the youngest children[5,6].

In light of current knowledge, asthma is a heterogeneous disease, which may include different, overlapping phenotypes. This heterogeneity made it necessary to establish different therapeutic strategies for the individual subtypes of asthma. Many clinical studies have been devoted to classifying patients with asthma according to phenotype, with the aim of improving the treatment of these patients and correlating the different pathophysiological airway processes[7].

Phenotype describes the clinical, physiological, morphological and biochemical features of a given disease, as well as the response to different therapies. The term "endotype" refers to the clinical picture and the underlying pathogenetic mechanism[8].

Allergic asthma, often co-occurring which other atopic diseases, predominates in the paediatric population. Positive skin tests, elevated titres of total immunoglobulin E, the presence of allergen-specific IgE, features of eosinophilic inflammation measured by induced sputum eosinophil count, and a positive family history of atopic diseases are also typical findings in allergic asthma. Patients usually respond well to treatment with inhaled corticosteroids (ICS). Due to the age-related differences in diagnostic and therapeutic approaches to asthma, the following paediatric age groups have been distinguished: young children aged ≤5 years (the so-called early childhood asthma), 6–11 years, and ≥12 years[9].

Biomarkers are biological indicators allowing for qualitative or quantitative assessment of various states, phenomena or biological features. They provide information about the pathophysiology and the course of the underlying disease, and can also predict patient's response to a given treatment, act as a marker of remission or disease severity. The measurement of biomarkers can accelerate the diagnostic process and allow for earlier initiation of appropriate therapy[10].

The molecular mechanisms underlying allergic asthma are closely related to the Th2-mediated immune response. Blood and sputum eosinophil counts, fractional exhaled nitric oxide (FeNO), serum IgE titres and periostin levels are among the most thoroughly investigated biomarkers of the Th2 pathway[11].

In the context of learning about new biomarkers, researchers' expectations particularly focus on the development of immunology and a more thorough understanding of pathophysiological processes. Progranulin (PGRN), a protein with anti-inflammatory and immunomodulatory properties, is a relatively new discovery.

Recent reports have confirmed a positive correlation between PGRN levels and pulmonary function among asthmatic patients, and therefore progranulin has been proposed as a new marker of asthma severity[12]. This molecule also induces proliferation of Treg cells and production of IL-10, which help maintain immune tolerance[13].

In recent years, data have also emerged on the role of matrix metalloproteinase-9 (MMP-9) in airway wall remodelling[14]. MMP-9 belongs to a family of proteolytic enzymes that contain zinc cations in their catalytic centre. Matrix metalloproteinases and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) play an important role in bronchial remodelling by affecting the function and migration of inflammatory cells and participating in extracellular matrix metabolism. MMP-9 is the predominant MMP in asthma, and its expression is enhanced during spontaneous exacerbations or in response to local instillation of allergen in the airway[15]. Its levels and/or activity are higher in asthmatic patients compared to healthy individuals[16].

Periostin is an extracellular matrix (ECM) protein belonging to the fasciclin family. Clinically, its levels are associated with bronchial remodelling and Th2-type inflammation[17]. Expression of the *POSTN* gene, which encodes periostin, is upregulated in bronchial epithelial cells by the major Th2-type cytokines IL-4 and IL-13. In the paediatric population, bone turnover can have a significant impact on serum periostin, hence its levels in children are two to three times higher than in adults[17].

Eosinophils are major effector cells in the pathophysiology of asthma and an important biomarker correlating with disease severity[18]. They are a central factor in Th2-driven inflammation and the predominant inflammatory cell type in the airways of children with severe asthma[19]. Although no standard cutoff value for eosinophilic inflammation has been set, a blood eosinophil

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count of about 300 cells/μL or a sputum eosinophil count >2–3% of the total cell count is used as a threshold[20,21]. Peripheral eosinophilia is considered a predictor of response to asthma treatment, immunotherapy in particular[22].

FeNO is considered a surrogate marker of eosinophilic airway inflammation. It is used to monitor the severity of airway inflammation and as a predictor of corticosteroid response[23].

An important role in asthma is attributed to regulatory T-cells (Tregs). They are essential in maintaining unresponsiveness to self-antigens and suppressing exaggerated immune responses to external antigens. Tregs secrete TGF-β and IL-10 and downregulate Th2 cells[24].

They are characterized by the expression of the transcription factor forkhead box protein P3 (FOXP3), which is probably responsible for their basic functions[25]. Asthmatic patients have been observed to have fewer peripheral Tregs[26]. It has therefore been suggested that a deficit and/or dysfunction of these cells may be related to the development of asthma[27].

MATERIALS

The study was conducted between May 2018 and August 2021. The study group included 25 asthmatic children aged 6–17 years (mean 10.5 ± 3.2 years), admitted to the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine – National Research Institute in Warsaw. There were 19 boys and 6 girls in this group. In all cases, asthma was diagnosed based on the GINA criteria. Children with confirmed atopy (elevated total IgE and/or positive skin prick tests) were included in the study. The patients were treated with inhaled GCs, antileukotrienes and beta2-adrenergic agonists at different doses; the use of inhaled GCs was a common feature and a mandatory criterion. Patients did not present features of airway infection at the time of the study, and the interval between the study and the last asthma exacerbation was >30 days.

The control group comprised 21 healthy children aged 6–18 years (mean 11.8 ± 4.2 years) with no history of allergic diseases. There were 14 boys (66.7%) and 7 girls (33.3%) in this group. No child presented with the symptoms of airway infection at the time of inclusion in the study; baseline laboratory findings were normal with low inflammatory markers. Children with other chronic conditions that could affect the results were not included.

The patients' parents were thoroughly informed about the purpose and course of the study and gave their informed written consent.

Initial qualification for the study was based on the author's questionnaire. Medical history was collected, and all children underwent a thorough physical examination and anthropometric measurements. Medical history included data on the onset of asthma, comorbidities, family history, previous diagnosis and treatment used.

Asthma control was assessed using the Childhood Asthma Control Test (C-ACT) for children under 12 years of age and the Asthma Control Test (ACT) for older children.

Based on the obtained score, patients were allocated to three groups:

1. **complete control of asthma**– ACT score of 25 or C-ACT score ≥19;
2. **partial control of asthma** – ACT score of 20–24 C-ACT score ≥19;
3. **poor control of asthma** – ACT score <20, C-ACT score <19.

ACT was developed by Nathan et al. for adults and children over 12 years of age. It consists of 5 questions rated 1–5. The score should be taken into account at the stage of treatment planning[28].

C-ACT was designed for younger children aged 4–11 years. It includes similar questions to those for older children, but there are only 7 questions, first 4 addressing the child, and the other 3 for the caregiver. A maximum score of 27 points can be obtained.

For the purpose of statistical analysis, the patients were allocated to two groups:

1. controlled asthma (good and partial asthma control);
2. uncontrolled asthma (poor asthma control).

METHODS

Fasting laboratory tests were performed in each patient. Two whole blood samples were used for blood counts with differential and Treg cell percentage analysis. The remaining blood tubes were centrifuged to obtain serum. C-reactive protein (CRP), total and specific IgE titres, as well as levels of PGRN, periostin, MMP-9 and Th1/Th2/Th17 profile cytokines were determined in the serum. Total IgE titres were measured using an immunoenzymatic (IE) assay. The sandwich enzyme linked immunosorbent assay (ELISA) was used to determine periostin, PGRN and MMP-9 levels. The percentage levels of Tregs and interleukin levels were measured using a cytometric method. The Medisoft FeNO device was used to measure FeNO levels. The results are presented in ppb. Spirometry was performed with the Lungtest 1000 MES device according to the current ATS (American Thoracic Society) and ERS (European Respiratory Society) guidelines and the position of the Polish Lung Association. The results of the study were subjected to statistical analysis. Statistical significance was set at $p < 0.05$.

RESULTS

The mean age was 10.5 ± 3.2 years in the study group and 11.8 ± 4.2 years in the control group. There were 6 girls (24%) and 19 boys (76%) in the asthmatic group,

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as well as 7 girls (33.3%) and 14 boys (66.7%) in the control group.

The mean age at asthma diagnosis was 6.8 ± 4.2 years. Other atopic diseases, such as allergic rhinitis (AR – 72%), atopic dermatitis (AD – 44%) and allergic conjunctivitis (16%), coexisted in 21 asthmatic children (84%). Nineteen children (76%) had a family history of atopic diseases. Skin tests for inhalant and/or food allergies were positive in all children. Elevated FeNO levels were found in 8 patients, accounting for 32% of the study group. The mean Tiffeneau index (FEV1%/VC) was 96.5% ± 9.7%.

Periostin levels were found to be statistically significantly higher in asthmatic children: 10,792.1 ± 5,089.4 ng/mL vs. 7,468.8 ± 4,812.5 ng/mL ($p = 0.03$) (Fig. 1).

There were no statistically significant differences between the study and control groups in terms of PGRN and MMP-9 levels (Tab. 1).

The pro-inflammatory cytokine **TNFα** showed higher levels in the asthmatic group (4.7 ± 6.3 pg/mL vs. 3.4 ± 8.7 pg/mL; $p = 0.039$). This difference was statistically significant (Tab. 1, Fig. 2).

Asthmatic children had significantly lower Treg percentages compared to controls (mean 2.5% ± 2.8% vs. 6.9% ± 0.3%, respectively; $p < 0.001$). The values are shown as a box-and-whisker plot in Fig. 3.

When analysing cytokine levels (Tab. 2), lower levels of pro-inflammatory cytokines were observed in the study group vs. controls. These differences were found for **IL-2** (3.5 ± 3.1 pg/mL vs. 5.7 ± 3.2 pg/mL; $p = 0.022$), **IL-6** (2.4 ± 2.2 pg/mL vs. 6.6 ± 3.2 pg/mL; $p < 0.001$), and **IFN-γ** (3.2 ± 2.9 pg/mL vs. 5.1 ± 2.0 pg/mL; $p = 0.017$), and were statistically significant.

The group of asthmatic children also showed statistically significantly lower levels of the Treg-secreted cytokine **IL-10** compared to controls (2.9 ± 2.4 pg/mL vs. 4.9 ± 2.8 pg/mL; $p = 0.014$) (Fig. 4).

Additionally, spirometry was performed, FeNO levels were measured and asthma control was assessed in the study group. Based on ACT and C-ACT findings, good, partial and poor asthma control was found in 4 (16%), 6 (24%) and 14 (56%) patients, respectively (Fig. 5). For the purpose of analysis, well-controlled and partially controlled asthma were considered as controlled asthma.

In controlled asthma, FEV1%/VC was higher than in uncontrolled asthma and were 99.8% ± 9.8% of the predicted normal value vs. 93.9% ± 9.1% of the predicted

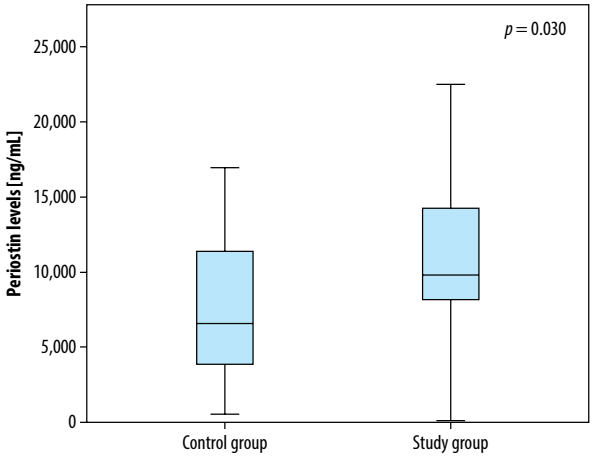

Fig. 1. Periostin levels in the study group vs. the control group

	Study group ($n = 25$)	Control group ($n = 21$)	p
Periostin [ng/mL], mean ± *SD*	10,792.1 ± 5,089.4	7,468.8 ± 4,812.5	**0.030**
PGRN [ng/mL], mean ± *SD*	57.5 ± 15.2	51.5 ± 9.8	0.132
TNFα [pg/mL], mean ± *SD*	4.7 ± 6.3	3.4 ± 8.7	**0.039**
MMP-9 [ng/mL], mean ± *SD*	336.9 ± 158.7	436.7 ± 208.0	0.076

Tab. 1. Comparison of selected asthma biomarkers between the study group and the control group

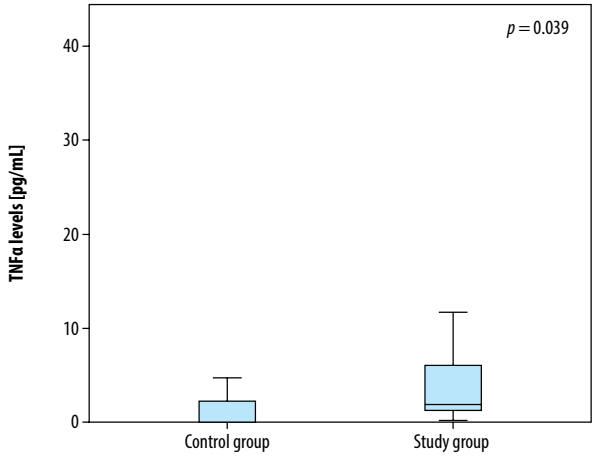

Fig. 2. TNFα levels in the study group vs. controls

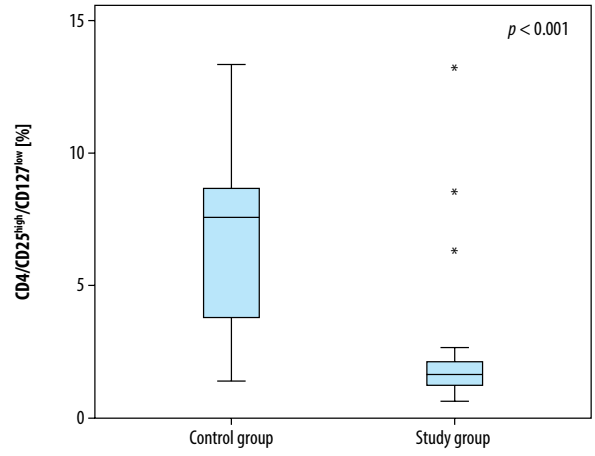

Fig. 3. Treg percentage in the study group vs. controls

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	Study group ($n = 25$)	Control group ($n = 21$)	p
Proinflammatory cytokines			
IL-2 [pg/mL], mean ± *SD*	3.5 ± 3.1	5.7 ± 3.2	**0.022**
IL-6 [pg/mL], mean ± *SD*	2.4 ± 2.2	6.6 ± 3.2	**<0.001**
IL-17 [pg/mL], mean ± *SD*	14.5 ± 15.6	18.8 ± 15.2	0.421
IFN-γ [pg/mL], mean ± *SD*	3.2 ± 2.9	5.1 ± 2.0	**0.017**
Anti-inflammatory cytokines			
IL-4 [pg/mL], mean ± *SD*	3.4 ± 3.2	5.2 ± 4.2	0.122
IL-10 [pg/mL], mean ± *SD*	2.9 ± 2.4	4.9 ±2.8	**0.014**

Tab. 2. Comparison of cytokine levels in the groups

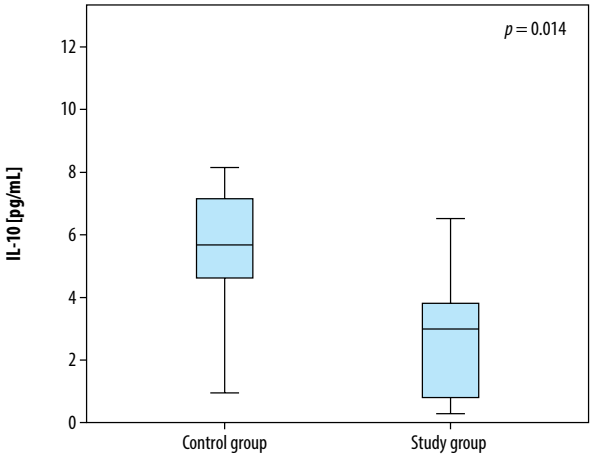

Fig. 4. IL-10 levels in the study group vs. controls

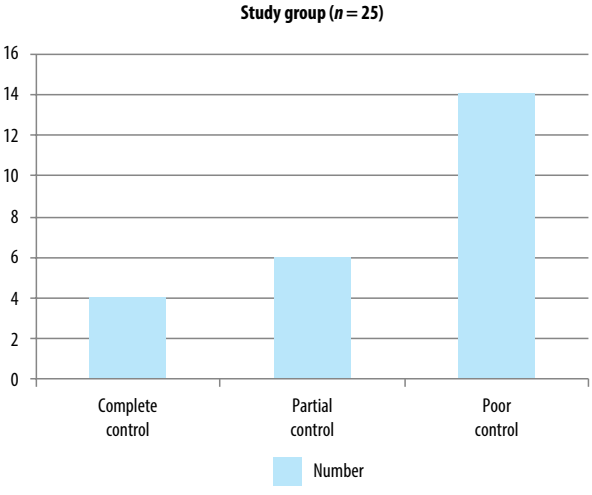

Fig. 5. Asthma control in the study group

normal value, respectively. FeNO levels were statistically significantly higher in uncontrolled asthma compared to controlled asthma (29.7 ± 22.9 ppb vs. 10.4 ± 4.2 ppb; $p = 0.008$) (Fig. 6).

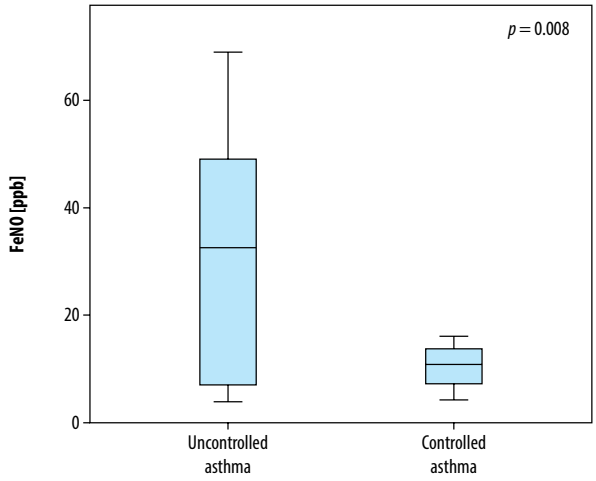

Fig. 6. FeNO levels in patients with controlled vs. uncontrolled asthma

	Controlled asthma ($n = 11$)	Uncontrolled asthma ($n = 14$)	p
Periostin [ng/mL], mean ± *SD*	9,667.8 ± 4,245.5	11,743.4 ± 5,699.6	0.330
PGRN [ng/mL], mean ± *SD*	55.7 ± 9.0	59.0 ± 19.3	0.617
TNFα [pg/mL], mean ± *SD*	5.4 ± 7.3	4.1 ± 5.6	0.612
MMP-9 [ng/mL], mean ± *SD*	306.8 ± 104.9	362.4 ± 193.8	0.383

Tab. 3. The levels of asthma biomarkers in patients with controlled vs. uncontrolled asthma

There were no statistically significant differences in periostin, PGRN, TNFα and MMP-9 levels between children with controlled vs. uncontrolled asthma (Tab. 3).

The group of asthmatic children with elevated FeNO showed statistically significantly higher PGRN levels than those with normal exhaled FeNO, i.e. 68.2 ± 14.7 ng/mL vs. 53.1 ± 13.5 ng/mL ($p = 0.024$) (Fig. 7). There was no statistically significant correlation between FeNO and other asthma biomarkers (Tab. 4).

There was a positive statistically significant correlation ($r = 0.535$, $p = 0.007$) between FeNO and serum PGRN (Fig. 8).

In the study group, children with eosinophil counts $>0.3 \times 10^3/\mu L$ showed higher FeNO levels (27.7 ± 22.3 ppb) compared to children with eosinophil counts $<0.3 \times 10^3/\mu L$ (11.5 ± 9.0 ppb) (Fig. 9). This difference was statistically significant ($p < 0.020$).

DISCUSSION

Most of the currently recognised asthma biomarkers available in clinical practice are associated with Th2-mediated inflammation. Predictive factors include for example FeNO, which now has a status of an eosinophilic

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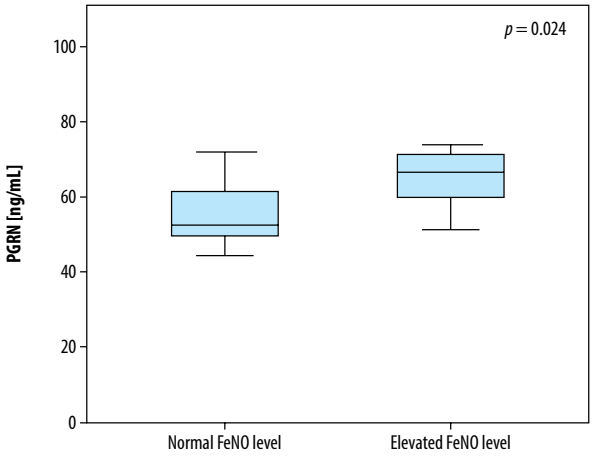

Fig. 7. PGRN levels in asthmatic children with normal vs. elevated FeNO

	Normal FeNO (*n* = 17)	Elevated FeNO (*n* = 8)	*p*
PGRN [ng/mL], mean ± *SD*	53.1 ± 13.5	68.2 ± 14.7	**0.024**
Periostin [ng/mL], mean ± *SD*	10,508.0 ± 4743.9	11,482.1 ± 6,204.4	0.680
MMP-9 [ng/mL], mean ± *SD*	323.4 ± 151.1	369.8 ± 184.1	0.527
TNFα [pg/mL], mean ± *SD*	5.3 ± 7.2	3.3 ± 3.7	0.475

Tab. 4. The levels of asthma biomarkers among asthmatic children with normal vs. elevated FeNO

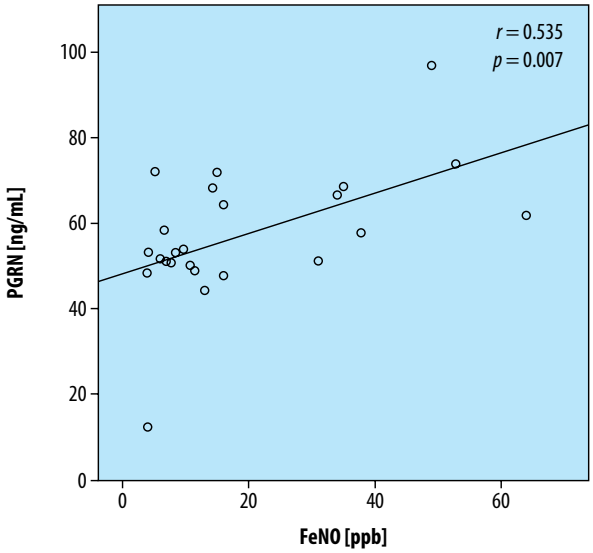

Fig. 8. A correlation between FeNO and PGRN levels

airway inflammation biomarker. According to available studies, FeNO measurement has contributed to improved prediction of asthma onset later in life[29]. Caudri et al. and Singer et al. showed that preschool children with higher FeNO values had a higher risk of developing asthma at school age[30,31]. Fang et al. demonstrated that FeNO

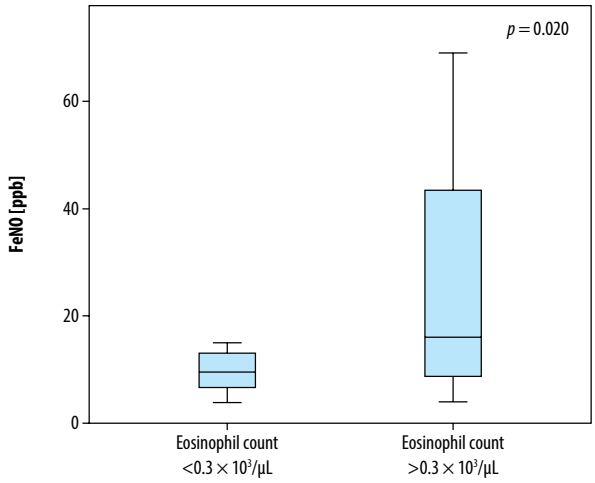

Fig. 9. FeNO in asthmatic children with eosinophil count <0.3 × 10³/μL vs. eosinophil count >0.3 × 10³/μL

levels correlate with bronchial hyperresponsiveness, as well as blood eosinophil counts and serum IgE titres in children[32]. FeNO measurement is used to identify children with atopic asthma who are likely to respond to GCs. Non-invasiveness is one of the main advantages of FeNO assessment, while the technical difficulties in performing the test and achieving cooperation in younger children are undoubted disadvantages. Elevated FeNO levels were found in eight children (32%) in our study group. Among the patients in this group, higher FeNO levels were found in children with uncontrolled asthma compared to those with good asthma control. This relationship was statistically significant. A correlation between FeNO values and blood eosinophil counts was also shown. The data support the usefulness of FeNO measurement in clinical practice.

Periostin is a protein that has gained biomarker status in Th2-mediated inflammation. It is induced by IL-4 and IL-13 and secreted by airway epithelial cells and lung fibroblasts[33]. Periostin is also secreted by periosteal cells and is involved in bone growth and repair, which is why it reaches higher levels in children than in adults[34]. Despite this limitation, children with asthma have been shown to have higher serum periostin levels compared to healthy children[35]. These observations were also confirmed in our study. Song et al. also demonstrated that higher serum levels of periostin are associated with bronchial hyperresponsiveness, higher blood eosinophil counts and higher IgE titres in asthmatic children[36]. A number of studies have also confirmed that periostin levels may be a marker of good response to IGC therapy[37]. Many attempts have been made to investigate the role of periostin in predicting the development of asthma. Anderson et al. conducted a prospective follow-up of children from the COAST (Childhood Origins of ASThma) cohort to investigate the role of several biomarkers of Th2-type inflammation: sensitisation to inhaled allergens, blood eosinophil counts and serum periostin levels in the development

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of childhood asthma. They found that, together with other variables, high serum periostin level at the age of 2 years was a risk factor for asthma by school age[38]. This relationship was not confirmed by a 2021 study in which Guvenir et al. investigated the role of periostin in young children with wheezing episodes for the prediction of asthma development. It was found that clinical features were more reliable than biomarkers in predicting the onset of asthma at school age in children with recurrent preschool wheezing[39]. There are no conclusive data to support the role of periostin as a potential biomarker of asthma development in children[40]. It also remains unclear whether periostin can be used as an asthma biomarker in the adult population. A project by Pavlidis et al. found that serum periostin levels are an insufficiently strong predictor of severe Th2-mediated asthma in adults and this is likely due to its production in the airway epithelium and its difficulty in reaching the systemic circulation[41]. Licari et al. published a study in which periostin levels were higher in asthmatic vs. healthy children, but they did not correlate with asthma severity and hence proved to be of limited utility in clinical practice[42]. In our study, serum periostin levels were found to be higher in asthmatic children compared to healthy controls, while no significant differences were observed in its levels in children with varying degrees of asthma control. These observations are consistent with recent reports. Recent meta-analyses have shown a significant and independent correlation between serum periostin levels and asthma severity in children; however the effectiveness of this parameter in identifying children with severe asthma was not satisfactory. Based on these results, it can be postulated that low serum periostin levels exclude severe asthma in children. Further studies are needed to determine the role of periostin in the pathogenesis of asthma and its clinical utility[43,44].

The study group was also shown to have higher levels of pro-inflammatory TNFα. Numerous studies have clearly demonstrated increased TNFα expression in patients with severe asthma[45,46]. Thomas and Heywood observed that inhaled TNFα, even in mild asthma, induced airway hyperreactivity associated with an increase in neutrophils and eosinophils[47]. TNFα undoubtedly plays a role in airway pathology, hence attempts to use this mechanism in the treatment. However, the results deviate from expectations. The use of anti-TNFα drugs in an adult population with moderate-to-severe asthma of undefined phenotype did not improve the course of asthma[48].

An impairment of the critical balance between Treg cells and pro-inflammatory lymphocytes in favour of the Th2 leads to the manifestation of asthma[49]. Currently, data on the presence of Treg cells in peripheral blood and bronchoalveolar lavage fluid (BALF) show reduced counts of CD4+CD25+FoxP3 cells in children with asthma compared to healthy children. These findings were obtained in patients not taking GCs[50,51]. Of interest is the phenomenon of an increase in serum and airway Treg levels after GC treatment[52]. Provoost et al. showed a reduced percentage of Foxp3-expressing cells within peripheral CD4+CD25^{high} lymphocytes in asthmatic patients; the mean percentage of these cells was 67.3% vs. 79.0% in the control group[27]. This is consistent with the findings reported by Agarwal et al. who found that the percentage of Foxp3+ cells in the peripheral CD4+CD25^{high} T-cell subpopulation in asthmatic patients was significantly reduced compared to healthy controls (49.00% vs. 95.91%, respectively)[53]. In our study, we found a significantly lower percentage of natural regulatory T cells (nTreg) and IL-10 in the serum of affected children compared to controls, confirming the role of these cells in the pathogenesis of asthma, which is consistent with the observations of other investigators.

MMP-9 was another potential asthma biomarker assessed in this study. This is a protein of the metalloproteinase family, produced mainly by macrophages and neutrophils, but also by epithelial cells, mast cells, fibroblasts and smooth myocytes. MMP-9 is a major mediator in bronchial wall remodelling and extracellular matrix metabolism[54]. Its increased levels were found in blood, sputum and bronchoalveolar lavage in patients experiencing asthma exacerbation[55]. Erlewyn-Lajeunesse et al. found that the ratio of MMP-9 to its natural inhibitor (TIMP-1) in BALF was higher in children with symptomatic asthma, as compared to that of healthy controls[56]. Furthermore, several independent studies have shown that MMP-9 deficiency in mice leads to airway inflammation, as well as an increased eosinophil count and cytokines such as IL-4 and IL-13[57]. In our study, MMP-9 levels were assessed by ELISA. No significant differences were found in the obtained results between asthmatic and healthy children. MMP-9 levels in both study groups did not depend on the degree of asthma control. It is possible that the results were influenced by the medications used or by the collection of specimens during the stable phase of the disease. Tanaka et al. found increased MMP-9 expression during spontaneous asthma exacerbations or in response to local allergen instillation. MMP-9 levels normalised after GCs and resolution of inflammation[15,58]. This highlights the need for further research on MMP-9 inhibition in the treatment of asthma.

PGRN is the cytokine proposed in this study as a potential biomarker for asthma. Its anti-inflammatory effects are, among other things, due to the inhibition of neutrophil degranulation by binding to TNFα receptors and thereby blocking TNFα–TNFR1/2 transmission. Additionally, it promotes the differentiation of naive T cells into Treg cells[59]. PGRN attenuates the course of TNFα-related inflammatory diseases, including respiratory diseases. This finding was confirmed by, among others, Ungurs et al. who, by analysing human airway sputum samples from patients with chronic obstructive pulmonary disease (COPD), demonstrated an

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inverse correlation between serum PGRN levels and the severity of neutrophilic airway inflammation[60]. Chen et al. showed that serum PGRN increases in patients with COPD and is higher in the exacerbation phase than in stable patients. Conclusions have been made that PGRN may act as both a COPD biomarker and an important factor involved in the pathogenesis of the disease exacerbation process[61]. In 2018, Chiba et al. published a study in which they demonstrated in a murine model (BALB/c) that intranasal administration of a recombinant PGRN preparation (Atsttrin) significantly inhibited bronchial hyperresponsiveness, probably by blocking the TNFα response. It was postulated, based on these results, that increasing the levels of PGRN may be a promising therapy for bronchial hyperresponsiveness in allergic asthma[62]. Similar findings were presented in 2021 by Liu et al., who produced a model chronic asthma in mice using dust mite (house dust mice, HDM). Recombinant PGRN was administered intranasally to mice during the HDM provocation phase. PGRN treatment was found to slow down the HDM-induced bronchial remodelling, as evidenced by inhibition of collagen accumulation, mucus overproduction, and airway smooth muscle synthesis in the lungs of HDM-treated asthmatic mice[63]. In contrast to these publications, however, Park et al. found that serum PGRN levels correlated negatively with the intensity of bronchial obstruction and reached significantly lower values in asthmatic patients from the study group than in healthy controls[12]. Although the exact mechanism underlying the anti-inflammatory effect of PGRN remains unknown, based on the literature review presented here, it appears that PGRN could be a potential marker of asthma severity and represents a promising object of research, also in terms of future therapeutic options. No studies on PGRN levels in asthmatic children were found in the available databases. This study is probably the first to assess this relationship. We showed that PGRN levels were higher in children with asthma than in controls, and that children with uncontrolled asthma had higher PGRN levels than children with controlled asthma. However, these differences were not statistically significant. This may be due to the fact that, according to the literature, PGRN showed elevated levels in asthmatic/COPD patients with neutrophilic inflammation. Only children with atopic asthma were included in our study. Also, we did not consider the effect of the treatment used in children. However, a positive, statistically significant correlation was found between PGRN levels and FeNO levels in children with asthma. Further studies and an attempt to compare PGRN levels in different phenotypes of this disorder are needed to draw further conclusions.

CONCLUSIONS

This study demonstrated the usefulness of serum periostin levels, FeNO and eosinophil percentage in the diagnosis of asthma in children, while the role of PGRN and MMP-9 remains debatable. Our findings and the cited publications demonstrate the need for further research on asthma biomarkers.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Author contribution

Original concept of study: MP. Collection, recording and/or compilation of data: MP. Analysis and interpretation of data: MP. Writing of manuscript: MP. Critical review of manuscript: BK, AR. Final approval of manuscript: BK.

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