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Biomarkers Reflecting the Severity of Bronchial Asthma in Children

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Background: Bronchial asthma, the most prevalent chronic inflammatory airway disease in children, exhibits a concerning rise in both incidence and prevalence. Asthma biomarkers hold promise for stratifying patients into distinct clinical phenotypes, paving the way for targeted and personalized treatment approaches.

Aim of Study: This study aimed to evaluate the association between novel and non-established semi-invasive circulating and well-known exhaled inflammatory biomarkers in two distinct pediatric asthma populations stratified by disease severity.

Materials and Methods: Forty-four asthmatic children aged 8–12 years meeting inclusion criteria were recruited from hospitalized patients. The first group (n=15, mean age 9.8 years) consisted of patients with mild persistent asthma who did not require regular inhaled corticosteroids (ICS). The second group (n=29, mean age 9.8 years) consisted of children with moderate to persistent asthma who received regular ICS treatment. Serum levels of interleukins (IL-13, IL-1β), eosinophil-derived neurotoxin (EDN), and surfactant protein D (SPD) were measured by ELISA in all participants. In addition, exhaled nitric oxide (FeNO) and blood eosinophil counts were evaluated.

Results: No significant differences were observed in the baseline plasma concentrations of inflammatory markers (IL-13, IL-1β, SPD, and EDN) or exhaled FeNO between the ICS-treated and non-ICS-treated groups. Further inter-individual analysis confirmed significant positive correlations between IL-13, SPD, and IL-1β (Pearson's r = 0.591–0.781) in both groups of patients. Interestingly, the ICS-treated group compared to the nontreated group showed an exclusive moderate negative correlation between FeNO and IL-1β. In contrast, FeNO exhibited a positive correlation with EDN and a strong association with eosinophil count in all the study groups.

Conclusion: Our findings highlight the complex and unresolved role of asthma biomarkers in routine clinical practice for the management of childhood asthma, particularly in predicting exacerbations. By comparing the relationships of carefully selected biomarkers, we can achieve a greater clinical predictive value.

Keywords: bronchial asthma, asthma biomarkers, children, childhood asthma, asthma severity

Introduction

Bronchial asthma is the most common chronic inflammatory disease of the airways that affects the pediatric population, with an increasing incidence and prevalence. Asthma is primarily characterized by symptoms, such as consistent wheezing, chronic cough, and difficulty in breathing. According to the GINA guidelines, more than half of adults with asthma experience their first symptoms during childhood.[1] This disease is highly diverse in terms of clinical presentation, course, and response to therapy. The vast majority of childhood asthma cases (>80%) are characterized by type 2 inflammation,[2] which is typically described by the predominance and activity of eosinophils and the polarization of the cellular response towards Th2 lymphocytes and innate lymphoid type 2 (ILC2) cells.

The term "endotype" describes disease clusters that are defined by distinct functional and pathophysiological mechanisms that underlie the onset of disease in a subset of patients with asthma.[3] Therefore, it is crucial to identify

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specific inflammasome cascades for asthma endotyping and targeted therapy. Asthma severity reflects how often and how severely symptoms affect patients' quality of life and lung function. Assessing asthma severity helps determine, evaluate, and adjust the appropriate treatment plan as well as identify and manage risk factors and triggers.[4] One way to enhance the diagnosis, classification, prognosis, and treatment of asthma is to identify biomarkers that show disease pathophysiology and severity.[5] Various biological sources provide biomarkers that are measurable indicators of biological processes or responses to intervention. Proper observation of biomarker oscillations can help to stratify patients with asthma into subgroups based on their endotypes and treatment targets. Furthermore, they can help to monitor disease activity, predict exacerbations, and evaluate the efficacy and safety of specific therapies.

In this study, we chose predefined asthma biomarkers that are involved in the development and persistence of the T2 high asthma endotype. Interleukin 13 (IL-13), interleukin 1 beta (IL-1β), surfactant protein D (SPD), and eosinophil-derived neurotoxin (EDN) are inflammatory mediators associated with bronchial asthma.[6] Figure 1 presents common definitions of the selected blood biomarkers according to their clinical values. As a non-invasive biomarker, we focused

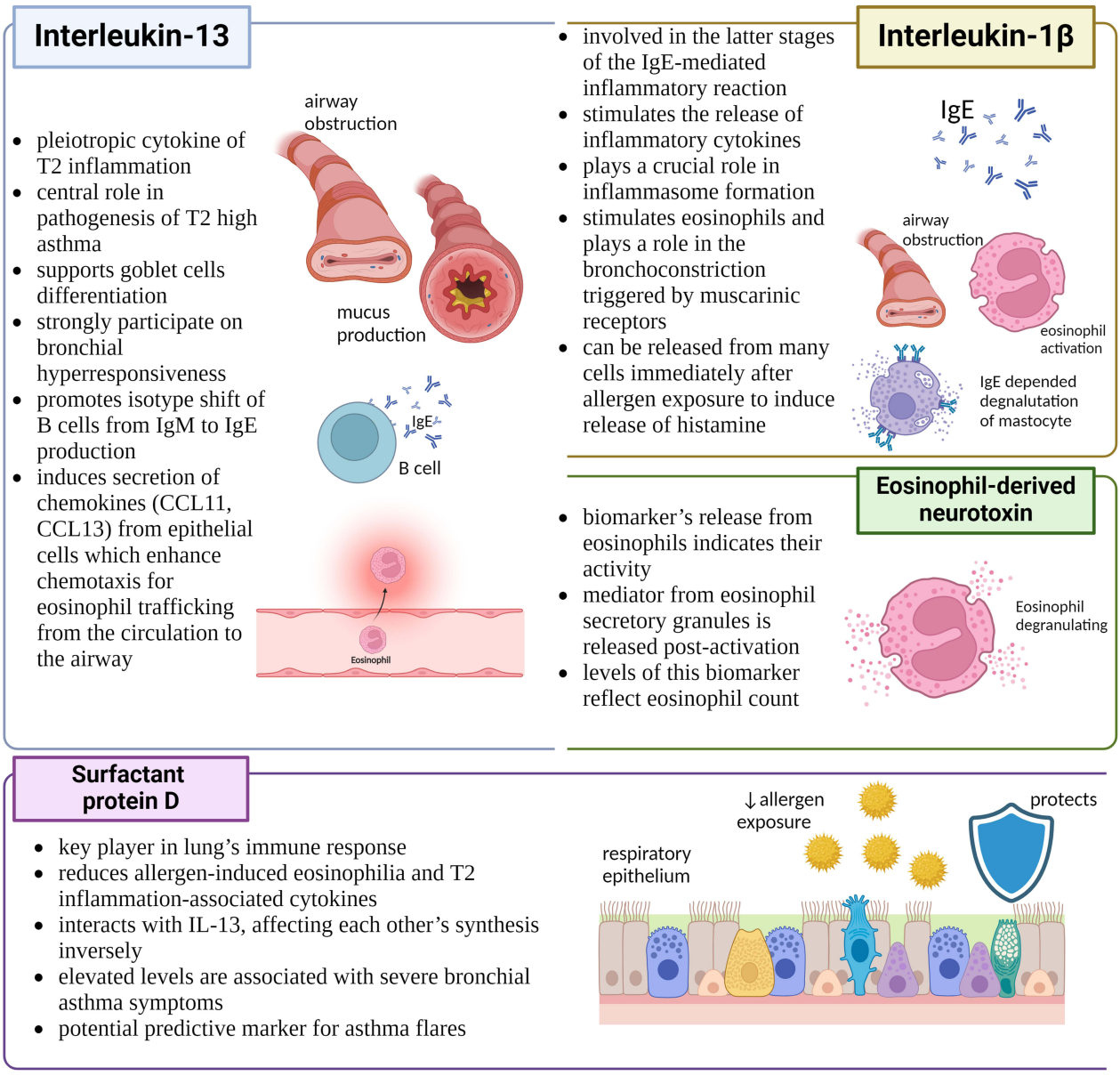

Figure 1 Main characteristics of selected biomarkers in relation with asthma pathogenesis, created in Biorender.com.

on the proportion of NO in exhaled air, which reflects the level of eosinophil bronchial inflammation. We investigated the association between the selected biomarkers in two distinct groups of patients based on disease severity.

Material and Methods

Study Population

This research was conducted at the National Institute of Pediatric Tuberculosis and Respiratory Diseases in Dolny Smokovec, Slovakia. In this study, 125 asthmatic patients aged between 8 and 12 years were selected from a pool of hospitalized individuals if they met the inclusion criteria. These criteria stipulated that the children must have been in good health for at least 14 days before their inclusion in the study, including not having been prescribed any antibiotic therapy. Furthermore, they should not have experienced any asthma flare-ups in the month without the use of a reliever, leading up to their inclusion in the study. The exclusion criteria were established to maintain the homogeneity of the study cohort. These criteria excluded subjects receiving immunomodulatory therapy, children who had been vaccinated within the last three months, and children on systemic corticosteroids. Additionally, children classified as obese (with a BMI exceeding the 97th percentile for their age) and patients with chronic diseases (such as cardiovascular diseases, severe congenital developmental defects, severe primary or secondary immunodeficiencies, malignancies, cystic fibrosis, bronchiectasis, or uncontrolled autoimmune diseases) were excluded from the study.

From the total number of patients, 15 children (9 boys and 6 girls; mean age, 9.8 ± 1.2 years) diagnosed and followed up with mild asthma were allocated to the first treatment group. Additionally, 29 children (mean age, 9.8 ± 1.3 years, consisting of 22 boys and 7 girls) with moderate to persistent asthma treated with inhaled corticosteroids were assigned to the second treatment group (Table 1). Written informed consent was obtained from the patient's parents/ legal guardians for participation in this study for all minor participants. Each proband underwent thorough medical history review and physical examination. Blood samples were obtained according to the manufacturer's guidelines for biomarker identification. All asthma-related medications were stopped for a minimum of 48 h before lung function tests were conducted. Spirometry tests were performed on the third day after admission.

Table 1 Fundamental Demographic, Anthropometric and Asthma Treatment Details of Studied Groups

	Group 1	**Group 2**
No.	15	29
Mean Age, y (range)	9.8 (± 1.2)	9.8 (± 1.3)
Male-to-female ratio	9:6	22:7
Weight, kg (range)	40.6 (31–49)	47.6 (30–65)
BMI, kg/m2 (range)	18.9 (12,5–24,1)	20.5 (15,5–24,8)
Height, cm (range)	139.6 (115–156)	146.2 (128–164)
Controller		
None	15	0
LTRA alone	3	0
ICS alone	0	12
ICS+LTRA	0	3
ICS/LABA	0	5
ICS/LABA+LTRA	0	10
OCS	0	0
Reliever (SABA on demand)	15	21
Antihistamine medication	7	25
C-ACT, points, (median)	25	22

Abbreviations: BMI, Body Mass Index; C-ACT, Children Asthma Control Test; LTRA, Leukotriene Receptor Antagonists; ICS, Inhaled Corticosteroids; LABA, Long-Acting Beta Agonists; OCS, Oral Corticosteroids,; SABA, short acting Beta Agonists.

The Ethics Committee of the National Institute of Pediatric Tuberculosis and Respiratory Diseases in Dolny Smokovec granted approval for this study, which was conducted as a prospective clinical control study. Investigations involving human participants were performed in accordance with the principles outlined in the 1975 Declaration of Helsinki, updated in 2013.

Subjects' Categorisation According Asthma Severity

The primary criterion for classifying subjects into the mild asthma group was the ability to maintain well-controlled disease without the need for persistent ICS administration coupled with satisfactory spirometry results. Mild asthma, as it is currently defined, is a condition that can be effectively managed with minimal treatment. This includes the use of on-demand low-dose ICS-formoterol or low-dose inhaled corticosteroids (ICS) in conjunction with as-needed SABA (as reliever). This approach reflects Steps 1 and 2 in the asthma treatment provided by the GINA guidelines. The second study group comprised patients who required a regular regimen of ICS (as a controller) either as monotherapy or in combination with SABA as needed, or ICS combined with long-acting beta-agonists (ICS-formoterol/salmeterol). This correlates with step 3–4 of the main asthma treatment track for children aged 5–11 years in GINA (Figure 2). This regimen is necessary to maintain optimal asthma control, which includes achieving satisfactory spirometry results.[1] All participants demonstrated effective asthma management, as evidenced by their adherence to the treatment protocols and sufficient skills in inhalation techniques. Asthma diagnoses were confirmed by specialists in clinical immunology, allergology, and pediatric pulmonology. Regular monitoring of patients was conducted in specialist outpatient clinics, with follow-up intervals typically ranging between 3–6 months.

Data Collection

Biomarker Measurement

The serum levels of IL-13, SPD, and IL-1β were determined using commercial ELISA kits (Sigma-Aldrich, USA). Similarly, we used an ELISA kit (Abbexa, UK) to measure plasma concentrations of EDN. For IL-13 and IL-1β evaluations, a known amount of standard protein (1pg/mL) was used to increase the levels of markers in samples with

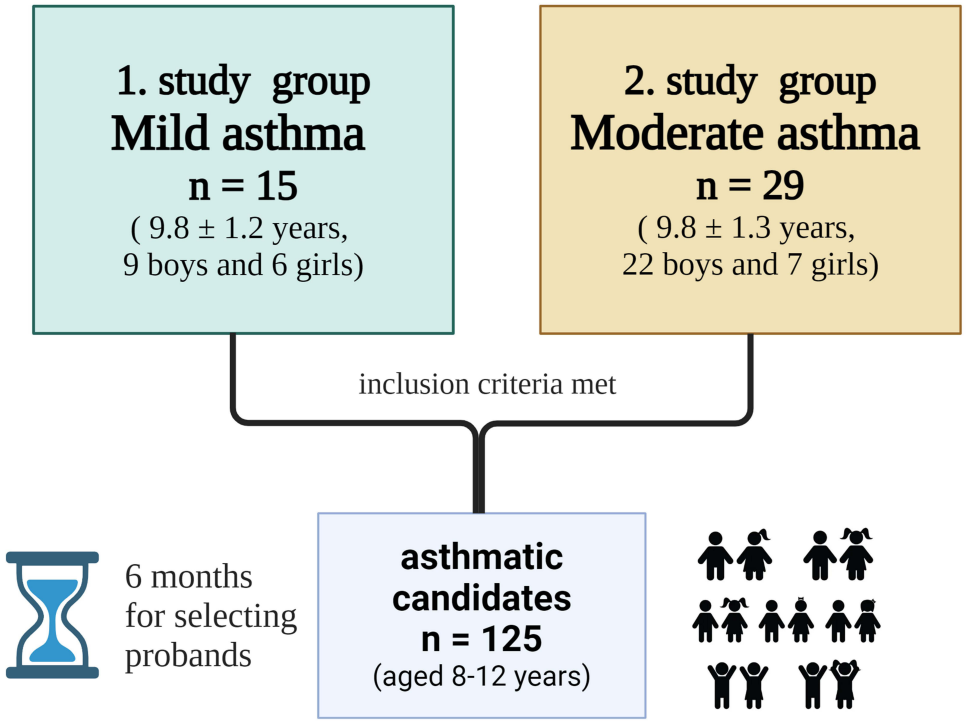

Figure 2 Flow chart of study population, created in Biorender.com.

insufficient concentrations. For the remaining two cytokines (SPD and EDN), we used a 2-time dilution of the obtained plasma samples. Colorimetric evaluation was performed using a Synergy 2 plate reader (BioTek, USA). Peripheral blood eosinophils were examined in absolute numbers (thousand/μL) as part of blood count parameters on a population haematology analyser (MINDRAY BC-5120 - Shenzhen Mindray Bio-Medical Electronics Co).

Spirometry, Bronchial Challenge Testing and FENO

Pulmonary function was evaluated using a professional spirometry device (Geratherm Respiratory GmbH). These tests were executed in strict compliance with the guidelines of the European Respiratory Society to ensure the validity and dependability of our research outcomes.[2,7] In line with the ERS task force guidelines, our study prioritized the following key spirometry parameters: FEV1, FVC, and FEV1 ratio, with a reduced FEV1/FVC ratio indicating airway obstruction. We set standard reference values to define the inclusion criteria of our study, requiring FEV1 and FEV1/FVC to exceed the lower limit of normal (LLN) or >80% of the predicted value. A positive bronchodilatation response was defined as an increase in FEV1 of ≥12% and/or ≥200 mL following inhalation of 400 μg of a short-acting β2-agonist for the assessment of bronchial lability, the hallmark of asthma.

Elevated levels of FENO (Fractional exhaled nitric oxide) serve as a reliable indicator of T2-type inflammation in the airways. The test results were obtained using a Niox Vero® electrochemical portable analyser, (Aerocrine AB, Solna, Sweden). Following inspiration, the children exhaled from their total lung capacity against a pressure ranging from to 5–20 cm H2O. This was facilitated by velopharyngeal closure to prevent nitric oxide contamination from the nasal cavity, thereby maintaining a constant flow of approximately 50 mL/s for 10s.[8] The outcome, expressed in parts per billion (ppb), was calculated as the mean of three distinct measurements. In addition to FeNO, patients exhibited other established biomarkers that are indicative of asthma. These included the total eosinophil count, cytological assessment of eosinophil count in nasal cilia, and the presence of total IgE antibodies. Collectively, these biomarkers contribute to a comprehensive understanding of asthmatic conditions in patients.

Statistical Analysis

The collected data were analyzed using Jamovi, a statistical software (The Jamovi Project, 2023, Sydney, Australia, Jamovi Version 2.3.28.0) [Computer Software], which was built in the R statistical language. The data predominantly exhibit high extremes. Consequently, we opted to employ the Shapiro–Wilk test, which confirmed the non-Gaussian distribution of data obtained from measurements of IL-1β, IL-13, SPD, and FENO levels. The measured factors, which were identified with a non-normal data distribution, were logarithmically transformed. To evaluate the statistical significance of differences between the study groups, we employed One-way Analysis of Variance (ANOVA). This method allowed us to discern any statistically significant differences between the means of the two groups. We also utilized a correlation matrix as a statistical method to analyze the relationship between the data acquired from the two study groups. The level of correlation between the study groups was assessed using Pearson's coefficient (r). The correlation was evaluated as very low when $0 < r < \pm 0.19$, low when $\pm 0.2 < r < \pm 0.39$, moderate when $\pm 0.4 < r < \pm 0.59$, strong $\pm 0.6 < r < \pm 0.79$, and as very strong when $\pm 0.8 < r < \pm 1$. The strength of significance was regarded as follows: p-value ranging from 0.05–0.01 corresponded to *0.01–0.001 corresponded to **and p-values lower than 0.001 corresponded to ***.

Results

Further analysis of the medical history and selected test results revealed distinct characteristics between the experimental groups. Patients receiving corticosteroids (group 2) exhibited a more pronounced atopic background, as confirmed by sensitization in both in vitro (21 vs 10) and in vivo (27 vs 13) tests. Conversely, corticosteroid-naive probands (Group 1) displayed higher mean total IgE (473.8 IU/L vs 252 IU/L) but almost similar peripheral blood eosinophil counts (374 vs 378 per μL). The significantly higher prevalence of systemic antihistamine use in Group 2 (25 vs 7) suggests a greater burden of allergy-related symptoms. Additionally, the higher risk of inadequate asthma control and asthma flares in group 2 could be potentially linked to a higher prevalence of smoking at home (13 vs 6) and pet exposure (21 vs 8).

Spirometry revealed mildly lower (FEV1% predicted: 85.23 vs 106.51), yet within the normal range, lung function in Group 2 children with moderate asthma. Group 2 also had a higher frequency of bronchial hyperreactivity (BHR) after

Table 2 Selected Clinical Attributes and Laboratory Findings of the Participants Included in the Study

	Group 1	Group 2
FENO (range)	20.7 p.p.b (0–107)	23.5 p.p.b (0–184)
Sensibilization on aeroallergens		
Skin prick test (in vivo test)	13	27
Specific IgE (in vitro test)	10	21
Total IgE, IU/l (range)	473.8 (10–3650)	252 (6–989)
Eosinophil count (per/μL)	374 (46–782)	378 (78–1262)
Eo in nasal epithelium (%, range)	7 (25–80)	10 (10–85)
Passive smoking exposure	6	13
Pets in households	8	21
Spirometry		
FEV 1, % predicted value (range)	106.51 (92–112)	85.23 (81–95)
FVC, % (range)	97.55 (86–120)	91.40 (85–105)
Positive (salbutamol) bronchial challenge test	9	14

Abbreviations: IgE, immunoglobulin E; FVC, forced vital capacity; FEV1, forced expiratory volume at the end of the first second of forced expiration; Eo, eosinophils.

salbutamol administration (14 vs 9) and a possible predisposition to airway mucosal dysbiosis based on nasopharyngeal swab analysis (18 vs 8). Table 2 summarizes the key clinical and laboratory findings.

Prior to performing a comparative analysis of the measured novel biomarkers, the normality of the data distribution was verified using the Shapiro–Wilk test. Most data exhibited a non-normal distribution. Exceptions were observed only in the distribution of the EDN values. These findings encouraged us to work with data processed by logarithmic calculations in subsequent statistical analyses.

A basic statistical comparison of the plasma concentrations of the selected markers, as well as exhaled FeNO, was conducted using ANOVA. This revealed no significant differences between patients treated with corticosteroids (group 2)

Table 3 Comparison of Differences Between Analysed Factors

	Group	Mean	SEM	P
FeNO	1	20.73	6.98	0.684
	2	23.52	6.58	
IL-13	1	51.45	10.63	0.711
	2	60.19	9.78	
IL-1B	1	28.4	10.53	0.169
	2	103.7	39.29	
EDN	1	8.06	0.6	0.231
	2	7.35	0.51	
SPD	1	63.27	53.17	0.519
	2	80.75	38.19	
Eosinophils	1	374	65.66	0.153
	2	375	52.89	

Note: Anova model for data obtained from two experimental groups. Values represents p.p.b in case of FeNO, abs. count/μL of blood in case of eosinophil number, pg/ μL of plasma in case of IL13, ng/mL in case of IL-1β, EDN and SPD. Corticosteroid naïve group 1 is compared to corticosteroid treated patient settled in group 2.

Table 4 Correlation Matrix, Corticoid Naïve Group (Group 1) and Corticoid Treated Group (group2)

Correlation Matrix – Group 1							
			IL-13	**IL-1B**	**EDN**	**SPD**	**Eo**
FeNO	Pearson's r	—					
IL-13	Pearson's r	0.294	—				
IL-1B	Pearson's r	−0.103	0.713**	—			
EDN	Pearson's r	0.302	0.023	−0.156	—		
SPD	Pearson's r	−0.135	0.518*	0.781***	−0.232	—	
Eosinophils	Pearson´s r	0.409	0.516	0.218	0.289	0.299	—
Correlation Matrix – Group 2							
		FeNO	**IL-13**	**IL-1B**	**EDN**	**SPD**	**Eo**
FeNO		—					
IL-13		−0.364	—				
IL-1B		−0.556**	0.591***	—			
EDN		0.361	−0.504**	−0.396*	—		
SPD		−0.371	0.634***	0.500**	−0.484**	—	
Eosinophils		0.803***	−0.327	−0.358	0.286	−0.374	

Note: Correlation analysis between measured inflammatory factors was performed separately in both experimental groups. Statistical significance is highlighted by * when p<0.05, ** when p<0.01 and by *** when p<0.001 by ***. Strength of correlation is depicted numerically by Person´s coefficient. Correlations of higher strength than moderate (Pearson´s >0.4) are highlighted by red colour.

and those who were corticosteroid-naïve (group 1) (Table 3). Logarithmic calculation had no impact on statistical significance observed in any of measured factors.

Further investigation was conducted to identify possible intraindividual associations between the measured markers. The results of the advanced correlation analysis applied to the calculated data confirmed positive correlations between IL-13, SPD, and IL-1β levels in both experimental groups. In the case of EDN, we observed a negative correlation between IL-13 and IL-1β exclusively in the corticosteroid-treated group.

Correlation analysis revealed a negative association between FeNO and IL-1β levels in young patients treated with corticosteroids. In the case of IL-13 and SPD, we have observed only weak correlation tendency with FeNO marker in corticosteroid treated group, without statistical significance. In contrast to IL-1β, IL-13, and SPD, FeNO was strongly positively correlated with eosinophil counts in the same experimental group (Table 4). Except for the positive correlation between markers IL-13, SPD, and IL-1β, there was no other association between the selected markers in the corticosteroid-naïve group of patients (Table 4).

Discussion

This study aimed to evaluate the association between selected circulating and exhaled inflammatory markers in two diverse groups of pediatric patients with bronchial asthma. The specific aim of this study was to compare two groups of patients, aged 8–12 years, who were either treated (n=29, Group 2) or not treated (n=15, Group 1) with inhaled corticosteroids.

In our study, we did not observe any significant differences in the inflammatory biomarkers (FeNO, IL-13, IL-1β, EDN, and SPD) between the two groups of subjects (p-values ranging from 0.169 to 0.719). This lack of significant

differences could potentially be attributed to the composition of each group of participants. Notably, the absence of patients with severe bronchial asthma refractory to conventional treatment may have influenced the results. In severe asthma, distinct clusters of biomarker positivity exhibit unique clinical characteristics, suggesting distinct patterns of underlying inflammatory pathway activation.[9] Our findings highlight the intricate and unresolved role of asthma biomarkers in routine clinical practice for the management of childhood asthma. Serum biomarkers, designed as less invasive alternatives to sputum analysis or bronchial biopsies, have demonstrated numerous limitations in terms of their clinical utility.[10] This highlights the surrogate characteristics of the selected biomarkers. Despite extensive research efforts, no new promising candidates have emerged from the vast array of innovative non-invasive serum biomarkers that show potential for additive application with commonly used assays, such as peripheral eosinophil count and FeNO.[11] Commonly available tests, such as total IgE, are frequently performed but have no significant predictive value. The sole exception is their utility in determining the indication and dosage of anti-IgE biological therapy.[6] Recently, periostin has garnered considerable attention as a promising new biomarker for type 2 asthma. While the initial results from selected clinical trials were encouraging, these findings were subsequently contradicted by a wealth of robust clinical data that did not support the utility of periostin in assessing asthma control.[12] Our findings emphasize the critical need for a deeper understanding of the intricate relationships between individual biomarkers and their comprehensive analysis. This knowledge gap necessitates further investigation of how novel biomarkers correlate with established clinical markers. Elucidating these associations is paramount for enhancing the clinical utility and relevance of these novel biomarkers. By evaluating a well-defined cluster of clearly delineated biomarkers in conjunction with the analysed interindividual characteristics, we may achieve a more precise assessment of asthma severity and exacerbation risk.

Many studies have associated serum biomarkers with asthma severity. Conversely, the effectiveness of serum biomarkers in assessing disease control or severity fluctuates greatly depending on the specific asthma population being studied. The observed variations could be traced back to a multitude of factors. These include differences in immune system reactivity among children due to age, a wide array of environmental influences, the absence of benchmark values, and significant variability between individuals. The cumulative effect of these factors poses considerable challenges for objective interpretation within the realm of clinical practice.[13–15] Type 2 inflammatory biomarkers, such as blood eosinophil levels and FeNO, have been identified as predictors of ICS response in children.[13,16] Genetic factors and non-invasive biomarker algorithms are emerging as important tools for optimizing ICS therapy, with the goal of maximizing efficacy while minimizing adverse effects. Biomarker assessment in this field in clinical practice demands more validation owing to the absence of adequate and pertinent scientific evidence. In the context of a therapeutic approach to asthma using ICS, biomarkers may not yield the anticipated influence necessary to optimize the evaluation of disease severity. A biomarker strategy using a composite score of T2 biomarkers did not result in a greater proportion of asthmatics reducing corticosteroid doses compared to a symptom-risk-based algorithm.[17] This conclusion supports our findings in each group, despite ICS treatment.

We found positive correlations between IL-13, SPD, and IL-1β levels in both groups. Numerous clinical investigations focusing on pediatric populations have underscored the practical value of these biomarkers in the administration of asthma and in assessing the potential for future exacerbations.[18–23] In our investigation of EDN, we discovered a negative correlation between IL-13 and IL-1β, a phenomenon unique to the patient group undergoing corticosteroid therapy. The presence of negative associations, especially between EDN and IL-13 levels, does not reflect the theoretical knowledge of their relationships. Eosinophils strongly contribute to asthma pathogenesis through the release of granule proteins such as EDN, which can cause airway injury and inflammation. Eosinophils from asthmatic patients contain higher intracellular concentrations of EDN than those from healthy individuals, indicating increased inflammatory capacity.[24] IL-13 is necessary for the induction of chemoattracted cytokines called eotaxins, which are involved in eosinophil recruitment, and IL-13-induced eotaxin-2 expression is critical for airway eosinophilia.[25] Higher serum IL-13 levels should be positively correlated with EDN release from eosinophils. This deduction presented in many theoretical articles was disturbed by data from the Phase III clinical trial of the anti-IL13 monoclonal antibody, lebrikizumab. Patients treated with lebrikizumab experienced increased blood eosinophil counts after 16 and 24 weeks of treatment. However, serum EDN levels remain unchanged.[26] This observation suggests a potential inverse relationship between EDN and IL-13, as a plausible explanation for our findings. Interestingly, IL-13 in the research of relationship with SARS-CoV-2 showed

potential of inhibition this infection in airway epithelial cells, potentially protecting individuals with T2 high asthma from COVID-19.[27] In relation to the connection between EDN and IL-1β, the limited references present in the scientific literature challenged our findings. Bronchoalveolar lavage fluid specimens obtained from individuals with mild allergic asthma following allergen challenge demonstrated a positive association with the measured quantities of IL-1β and EDN.[28]

FENO, a suitable T2 high asthma biomarker in children, reflects eosinophil inflammation in airways. IL-13 upregulates the expression and transcription of mediators in the signalling pathways linked to type 2 inflammation. This upregulation promotes the formation of inducible nitric oxide synthase (iNOS), leading to the production of nitric oxide (NO). NO, a small, freely diffusible molecule, readily traverses cell membranes and reaches bronchi.[29,30] This study corroborates the weak negative correlation between FeNO and IL-1β levels in asthmatic patients receiving corticosteroid therapy. These findings align with research demonstrating similar negative associations between these parameters during the investigation of their potential in asthma assessment in obese patients.[31] Concordant findings were reported in another study investigating the association between IL-1β gene polymorphisms and future asthma risk in children. Likewise, a post-hoc analysis within this study revealed no significant positive correlations between serum IL-1β levels, FeNO concentrations, and lung function.[23] Clinical studies have revealed that serum EDN is a more reliable indicator of asthma control status than blood eosinophil count.[32] The results in both groups demonstrated a tendency of significant positive correlation between FeNO and EDN. The observed positive correlation (on the edge significance) between FeNO and eosinophilic degranulation products (eg, EDN) levels in asthmatics aligns with the known association between eosinophilic airway inflammation and NO overproduction.[33] This link has been supported by findings from several clinical studies. One study showed a positive correlation between EDN measured in plasma, serum, and sputum with FeNO values in asthmatics with an aspirin-exacerbated asthma phenotype.[34] The investigators of the second study assessed the combined utility of EDN and FeNO alongside other T2 inflammatory biomarkers in severe uncontrolled and difficult-to-treat asthma. This study aimed to identify potential cut-off values for predicting the risk of disease flares. Notably, subjects with FeNO levels exceeding 25 ppb displayed a directly proportional increase in serum EDN concentration.[35] The well-established association between FENO levels and blood eosinophil counts makes them crucial biomarkers for selecting patients with moderate asthma who may benefit from biologics that block the T2 inflammatory pathways.[36–38] This finding was further supported by our confirmation of a strong association between these biomarkers in children with moderate asthma.

The primary constraints of this clinical study are the limited number of subjects, narrow range of biomarkers examined, and exclusion of pediatric patients with severe bronchial asthma. We postulate that these factors may have contributed significantly to the minimal statistical significance observed among the studied variables. Future investigations should consider these factors when defining the research design and objectives. The principal merit of our study lies in its comprehensive research approach, which aimed to explore the potential supplementary role of selected T2 biomarkers of asthma endotypes in distinguishing various forms of asthma severity and predicting the risk of exacerbation. In particular, there was a close positive relationship between IL-13 (Th2 and ILC2 derived cytokine) and IL-1β (Th1/Th17 and ILC3 derived cytokine) in both groups. These observations point at heterogenous and yet not fully described pathogenesis of asthma with possibility of pathways overlap.

Conclusions

The diagnosis and monitoring of bronchial asthma currently rely primarily on evaluating the dynamics of symptom intensity and frequency. Clinically applicable biomarkers of asthma could offer significant advantages by serving as indirect indicators of the extent and nature of the inflammatory activity within the tracheobronchial tree.

In current clinical practice, only a few biomarkers are used to assess and monitor bronchial inflammation, with limited benefits. In the era of personalized medicine, asthma biomarkers represent a crucial effort to stratify patients based on different asthma subtypes, thereby enabling the development of targeted and specific therapeutic approaches. Current research has focused on identifying biomarker panels to improve the management of childhood asthma. However, as our results and the broader field acknowledge, substantial work remains for clinical validation and widespread adoption of novel biomarkers in routine clinical practice.

Data Sharing Statement

The datasets analyzed during the current study are available in the cloud center (Microsoft One Drive) repository at: Biomarkers reflecting the severity of bronchial asthma in children_datasets.xlsx

Ethics Statements

For all participants under the age of 18, written informed consent was obtained from their parents/legal guardians. The study was approved by the Ethics Committee of the National Institute of Pediatric Tuberculosis and Respiratory Diseases in Dolny Smokovec, Slovakia, and conducted as a prospective clinical control trial. All investigations involving human participants adhered to the ethical principles established in the Declaration of Helsinki (1975, revised 2013).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to declare.

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