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# Cytokine profile in childhood asthma

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**ABSTRACT**

Childhood asthma is a chronic airway disease, which pathogenesis is markedly heterogeneous–with multiple phenotypes defining visible characteristics and endotypes defining molecular mechanisms. Cytokines and chemokines released during inflammatory responses are key immune mediators. The cytokine response can largely determine the susceptibility to childhood asthma and its severity. The purpose of this study was to characterize the immune profile of childhood asthma. The study involved 26 children (3–18 years old), who were divided into 2 groups: study–with childhood asthma; control–without asthma. The innovative Bio-Plex method was used to determine the serum concentration of 37 inflammatory proteins in one experiment. The results were analyzed using univariate statistical tests. In the study group, the level of the 10 tested markers increased, while the level of the remaining 9 decreased compared to the control; a statistically significant reduction in concentration was obtained only for the MMP-1 ($p < 0.05$). According to the ROC curve, MMP-1 can be considered an effective discriminator of childhood asthma ($p < 0.05$; AUC = 0.752). Cytokines/chemokines may be useful in the diagnosis of childhood asthma and may also become a prognostic target in determining the phenotype/endotype of this condition. This study should be a prelude to and an incentive for more complex proteomic analyzes.

## Introduction

Childhood asthma has become a public health problem worldwide [1]. The global prevalence of asthmatic symptoms among 6-7-year-olds is 11.5% and 13-14-year-olds 14.1% [2]. Moreover, the number of children suffering from asthma continues to grow [3]. In the face of this situation, the most important thing is to improve and optimize diagnostic and therapeutic procedures. Currently, the correct, early diagnosis of asthma in children constitutes a huge challenge. Symptoms of childhood asthma may be atypical or overlap with other respiratory diseases characterized by obstruction of the airways [4]. The criteria used for diagnosis may also vary according to different guidelines, studies, and among clinicians. Furthermore there is difficult to perform objective pulmonary function tests at such an early age [4]. Diagnosis, as well as predicting response to treatment, may be made easier by recognizing childhood asthma phenotypes/endotypes, that is, the underlying pathophysiological and/or molecular mechanisms [5]. There are significant differences between childhood and adult asthma regarding immunology, histopathology, and clinical symptoms. Many researchers are still trying to identify specific biological markers of childhood asthma that would assist in the diagnosis of this disorder. Finding these compounds is extremely important as accurate diagnosis and optimal treatment play a key role in the proper functioning of children with asthma and can significantly improve their quality of life [6]. Currently, there is still a need to search for new, more precise, and accurate diagnostic methods, differentiating phenotypes and endotypes of asthma so that a complete, personalized approach to the patient will be possible.

Asthma is characterized by immune system activation, airway hyperresponsiveness (AHR), epithelial cell activation, mucus overproduction and airway remodeling. The immune system is thought to be a regulator of asthma and airways inflammation. Both innate and adaptive immunity play roles in immunologic mechanisms of asthma [7]. Important mediators of immunity are cytokines and chemokines [8]. They are secreted during inflammatory response [9]. Cytokines regulate the production of other inflammatory mediators and chemokines attract leucocytes to the site of inflammation [9]. The cytokine response due to an imbalance or deficiency in the cytokine network can largely determine the susceptibility to childhood asthma and its severity. Inflammatory factors may be useful in identifying chronic airway inflammation in childhood asthma and could be a promising prognostic target in phenotype/ endotype differentiation [9]. Many cytokines are involved in asthma pathogenesis, including: interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8; CXCL8), interleukin-18 (IL-18), C-C motif chemokine ligand 3 (CCL3), macrophage migration inhibitory factor (MIF), C-C motif chemokine ligand 5 (CCL5; RANTES), interferon gamma-induced protein 10 (IP10; CXCL10), tumor necrosis factor α (TNF-α) and interferon gamma (IFN-g) [9–11]. Furthermore circulating levels of proteins are dynamic and modifiable and therefore amenable to therapeutic targeting [8].

## Aim

The aim of this study is to characterize the cytokine profile of childhood asthma by determining the serum concentrations of 37 circulating inflammatory factors, which are probably involved in the pathogenesis of this condition. Our research findings could be helpful in the search for new biomarkers and therapeutic targets for childhood asthma. By learning more biomarkers, it will be possible to diagnose, monitor and adjust treatment of asthma. The use of the Bio-Plex system enabled the study of thirty-seven inflammatory factors in one experiment. This method quantified a biologically relevant target using fluorescently dyed magnetic beads [12].

## Materials and Methods

### Patients and Serum Samples

Twenty-six participants aged 3-18 were qualified for the experiment and divided into two groups. The study group consisted of children with asthma (n = 11) and the control group of children without asthma and allergies (n = 15). The control group was appropriately matched to the study group in terms of gender, age, and ethnicity. **Table 1** presents the characteristics of patients from the asthma group and the control

**Table 1.** Characteristics of patients with asthma (study group) and without asthma and allergies (control group)

| Characteristics of the Participants *(n = 26)* | | Asthma (Study Group) *(n = 11)* No (%) | No Asthma (Control Group) *(n = 15)* No (%) |
|---|---|---|---|
| Age | | | |
| – Median<br>– Range | | 12<br>4-16 | 10<br>3-18 |
| Sex | | | |
| – Female<br>– Male | | 5 (45.5)<br>6 (54.5) | 6 (40)<br>9 (60) |
| Asthma Severity | | | |
| – Mild<br>– Moderate<br>– Severe | | 5 (45.5)<br>4 (36.3)<br>2 (18.2) | |
| Budesonide or Equivalent (The daily dose of GCs) | | | |
| – 100 – 200 µg<br>– 250 – 350 µg<br>– 400 – 500 µg<br>– >500 µg<br>– No information | | 4 (36.3)<br>2 (18.2)<br>3 (27.3)<br>1 (9.1)<br>1 (9.1) | |
| Comorbidities | | | |
| – Atopic Dermatitis<br>– Hypoacusia<br>– Allergic Rhinitis<br>– Cholecystitis<br>– Coeliac Disease | | 1<br>1<br>3<br>1<br>1 | |
| Total IgE (kU/l) | | | |
| – Mean<br>– Range | | 278.61<br>15.5-1035 | 96.29<br>12.2-797 |
| Lung Function (%) | | | |
| – FEV1/VC | Mean<br>Range | 85.25<br>67-94 | |
| – FEV1 | Mean<br>Range | 82.5<br>40-97 | |
| – FVC EX | Mean<br>Range | 87-33<br>49-95 | |

group. The recruitment of children was carried out through the Department of Pediatric Pneumonology, Allergology and Clinical Immunology, K. Jonscher Clinical Hospital of the Medical University in Poznan, after prior written consent from the guardians of the children. The material for the research was obtained by collecting blood from patients into appropriate test tubes. The blood was centrifuged to obtain serum samples. The test material was properly secured and stored at -80°C until analysis. The study was approved by the Local Ethical Committee of the Medical University of Poznan, Poland (Decision No. 530/12), in accordance with the requirements of the Helsinki Declaration.

## Inflammatory Marker Panel Measurement

The experiment involved the simultaneous determination of thirty-seven proteins of the inflammatory response (a proliferation-inducing ligand/ tumor necrosis factor ligand superfamily member 13 (APRIL/TNFSF13), B-cell activating factor/ tumor necrosis factor ligand superfamily member 13B (BAFF/TNFSF13B), soluble form of CD30/ tumor necrosis factor receptor superfamily member 8 (sCD30/TNFRSF8), the macrophage activation marker-soluble CD163 (sCD163), Chitinase-3-like 1, gp130/sIL-6Rβ, interferon alpha-2 (IFN-α2), interferon β (IFN-β), interferon γ (IFN-γ), IL-2, sIL-6Rα, IL-8, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-19, IL-20, IL-22, IL-26, IL-27 (p28), IL-28A/

IFN-λ2, IL-29/IFN-λ1, IL-32, IL-34, IL-35, tumor necrosis factor superfamily member 14 (LIGHT/TNFSF14), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, thymic stromal lymphopoietin (TSLP), TNF-related weak inducer of apoptosis/tumor necrosis factor ligand superfamily member 12 (TWEAK/TNFSF12)) using the Bio-Plex Pro Human Inflammation Panel 1 assay (Bio-Rad, Hercules, CA, USA), in accordance with the instructions included in the manufacturer's leaflet. The inflammatory response protein profile was determined in serum using the following methods: flow cytometry, magnetic separation. The kit contains reagents, including standards and quality controls, and a reaction site, i.e., a 96-well plate. The principle of the Bio-Plex method is based on the use of primary antibodies conjugated with fluorescent magnetic beads, which have different colors and are directed against targeted markers. Briefly, 50 microliters of the test material, reagents (standards and quality controls) were added to each well of the plate containing the primary antibodies attached to the beads, and then prepared mixture was incubated for one hour at room temperature. Upon completion of incubation and subsequent sample washing cycles, antibody-biotin reporters for detection were inserted into the wells. Preparation of the final reaction mixture was accomplished by adding the streptavidin-phycoerythrin fluorescent conjugate. The concentration of inflammatory response proteins was measured by flow cytometry using a Bio-Plex array reader (Bio-Plex MAGPIX, Bio-Rad, Hercules, CA, USA). The reader is equipped with two LEDs, one of which emits red light with a wavelength of 635 nm and the other, which in turn emits green light with a wavelength of 532 nm. The obtained data was pre-processed with Bio-Plex Manager 6.0 software (Bio Rad, Hercules, CA, USA). Before the analysis, the software was completely calibrated and verified. The standard curve was created based on the standards provided by the manufacturer. The concentrations of the determined markers were presented in picograms per milliliter (pg/ml) analogously to the standard curves. Two of the 96 wells of the plate were filled with Bio-Rad diluents only, which were interpreted as blank. Negative and positive quality controls were used to verify that the procedure was correctly performed.

### Data Analysis

Statistica 13.0 (StatSoft Inc., Tulsa, OK, USA) and MedCalc (MedCalc Software Ltd, Ostend, Belgium) were used for statistical analysis. Data with $p < 0.05$ were considered statistically significant. The obtained values were analyzed using univariate statistical tests. Depending on the type of data distribution, a detailed comparison of the control group with the study group was carried out using the t-test or Mann-Whitney test. The Shapiro-Wilk test was used to check the normality of the data distribution. Variables without a normal distribution were compared with the Mann-Whitney U test, and the equality of variance for normally distributed variables was tested with Levene's test. If the result obtained with Levene's test was statistically insignificant ($p > 0.05$), the variance between the groups was homogeneous and the Student's t-test was performed successively. If the Levene's test result was statistically significant ($p < 0.05$), the Welch t-test was performed. The univariate receiver operational characteristic (ROC) curve was determined by MedCalc (MedCalc Software Ltd, Ostend, Belgium). The visual correlation between sensitivity and specificity is presented on the ROC curve for each of the analyzed analytes. The ROC curve allows the specificity and sensitivity of the discriminator to be assessed.

## Results

### Serum Profile of Inflammatory Proteins in Childhood Asthma.

The Bio-Plex system used in the experiment allowed for the simultaneous measurement of the concentration of multiple cytokines and chemokines of the inflammatory response in the serum during one experiment. Successful measurement was performed in the serum of nineteen out of 37 (APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, sIL-6Rα, IL-19, MMP-1, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP, and TWEAK/TNFSF12) analyzed inflammatory factors. The results obtained in both groups subjected to the study are presented in **Table 2** and **Figure 1**. The remaining eighteen inflammatory markers were eliminated from further data analysis because the obtained concentrations were below the quantification limit or

**Table 2.** The values of the concentrations of 19 proteins of the inflammatory response in the study and control groups. The results are given in pg/ml

| Inflammation Marker | Asthma (Study Group) | | | | | No Asthma (Control Group) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Median | Q1 | Q3 | Mean | SD | Median | Q1 | Q3 | Mean | SD |
| APRIL/TNFSF13 | 12762,1 | 7915,8 | 32639,5 | 19870,8 | 17420,8 | 17734,5 | 14481,4 | 21877,5 | 19149,4 | 10960,5 |
| BAFF/TNFSF13B | 9609,6 | 7247,1 | 10911,4 | 9215,0 | 2279,8 | 8732,1 | 7448,5 | 9740,4 | 9459,0 | 3673,5 |
| sCD30/TNFRSF8 | 1448,1 | 1172,8 | 1727,3 | 1410,5 | 408,0 | 1475,4 | 1000,0 | 1892,4 | 1507,8 | 721,1 |
| sCD163 | 146695,1 | 142626,0 | 221083,1 | 169391,4 | 57001,1 | 138355,9 | 103637,3 | 187489,5 | 156009,9 | 72201,3 |
| Chitinase 3-like 1 | 6037,7 | 3729,6 | 9393,0 | 6685,6 | 3103,5 | 6790,3 | 4652,3 | 10186,1 | 7193,0 | 2805,4 |
| gp130/sIL-6Rbeta | 60735,9 | 56092,1 | 70486,7 | 67854,5 | 16322,3 | 62216,7 | 40731,1 | 73583,0 | 61963,6 | 22688,7 |
| sIL-6Ralfa | 15817,9 | 11713,8 | 17580,4 | 15141,8 | 3444,9 | 12329,2 | 10746,9 | 16614,7 | 12776,1 | 4444,6 |
| IL-19 | 70,9 | 61,5 | 79,9 | 70,0 | 11,8 | 68,0 | 57,9 | 75,4 | 66,5 | 18,2 |
| IL-26 | 92,5 | 83,1 | 107,1 | 94,2 | 20,1 | 92,5 | 61,6 | 133,5 | 96,6 | 36,7 |
| MMP-1 | 901,7 | 695,7 | 1160,8 | 1056,5 | 583,5 | 1686,9 | 1105,1 | 2373,0 | 2585,6 | 3402,7 |
| MMP-2 | 36789,1 | 27049,2 | 50823,7 | 40098,6 | 17277,9 | 29532,6 | 19322,0 | 43173,5 | 37722,8 | 26801,2 |
| MMP-3 | 2169,8 | 726,2 | 3381,3 | 3675,0 | 5911,6 | 2045,3 | 1444,2 | 2134,9 | 2097,5 | 1099,2 |
| Osteocalcin | 17120,7 | 11015,3 | 20686,4 | 16445,1 | 5195,2 | 12019,2 | 7979,1 | 19130,9 | 14598,1 | 9154,6 |
| Osteopontin (OPN) | 33548,8 | 30052,6 | 36918,0 | 33919,2 | 6745,6 | 41168,1 | 16608,5 | 46928,8 | 36610,2 | 16446,9 |
| Pentraxin-3 | 1099,8 | 612,4 | 1652,1 | 1167,5 | 648,2 | 866,2 | 659,7 | 1646,9 | 1257,0 | 877,3 |
| sTNF-R1 | 3227,8 | 2812,9 | 4835,3 | 3682,8 | 1110,9 | 3479,1 | 2619,0 | 4635,8 | 3849,9 | 1619,5 |
| sTNF-R2 | 5635,6 | 3823,4 | 7457,1 | 6021,3 | 2564,4 | 5294,8 | 2780,5 | 6963,5 | 5433,9 | 3405,1 |
| TSLP | 21,3 | 17,3 | 26,5 | 21,9 | 6,4 | 22,5 | 20,9 | 22,5 | 22,7 | 4,5 |
| TWEAK/TNFSF12 | 678,4 | 535,6 | 757,4 | 642,7 | 139,8 | 535,6 | 421,2 | 749,5 | 590,2 | 212,8 |

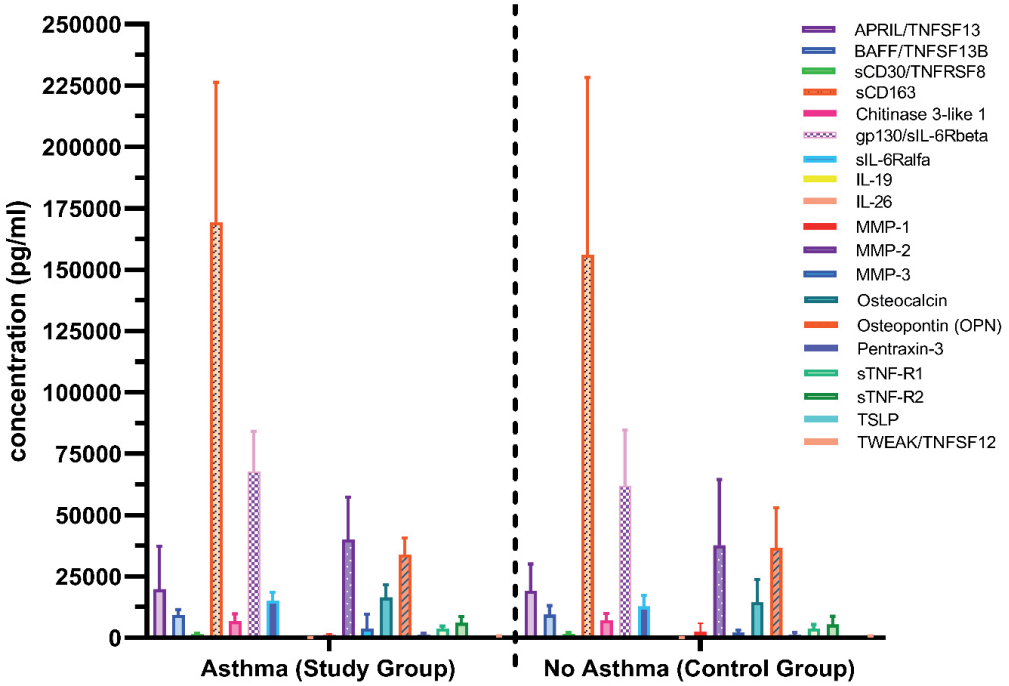


**Figure 1.** Comparison of the concentration of inflammatory proteins in the serum between the two groups: childhood asthma (study group) and control group

were incomplete. Ultimately, our statistical analyzes included 19 inflammatory response proteins for which the data were complete.

The inflammatory marker profile of patients with childhood asthma (study group) was compared to non-asthma and allergy-free volunteers (control group) using the Student's t-test, Mann-Whitney or Welch test. The most important data from these comparisons are presented in **Table 3**. After performing univariate statis-

**Table 3.** Univariate statistical analysis of serum inflammatory response proteins in children with and without asthma. The numerical values of "p" indicate statistical significance

| Inflammation Marker | p value |
|---|---|
| | Mann-Whitney U Test |
| APRIL/TNFSF13 | 0,436 |
| BAFF/TNFSF13B | 0,568 |
| sCD30/TNFRSF8 | 0,876 |
| sCD163 | 0,253 |
| Chitinase 3-like 1 | 0,678 |
| gp130/sIL-6Rbeta | 0,436 |
| sIL-6Ralfa | 0,194 |
| IL-19 | 0,775 |
| IL-26 | 0,815 |
| MMP-1 | 0,033 |
| MMP-2 | 0,233 |
| MMP-3 | 0,979 |
| Osteocalcin | 0,324 |
| Osteopontin (OPN) | 0,35 |
| Pentraxin-3 | 0,876 |
| sTNF-R1 | 0,917 |
| sTNF-R2 | 0,467 |
| TSLP | 0,484 |
| TWEAK/TNFSF12 | 0,311 |

tical tests, a statistically significant difference was obtained between the studied groups in the concentration of one inflammatory factor. In the group of children with asthma, the circulating level of MMP-1 was significantly decreased ($p < 0.05$) compared to the control group. Other inflammatory factors were also characterized by differences in concentrations between the study and control groups, but the observed differences were not statistically significant. There was a slight increase in the concentration of 10 inflammatory factors (APRIL/TNFSF13, sCD163, gp130/sIL-6Rbeta, sIL-6Ralfa, IL-19, MMP-2, MMP-3, Osteocalcin, sTNF-R2, TWEAK/TNFSF12) in the group of children with asthma compared to control. On the other hand, the concentration of the remaining 8 inflammatory factors (BAFF/TNFSF13B, sCD30/TNFRSF8, Chitinase 3-like 1, IL-26, Osteopontin (OPN), Pentraxin-3, sTNF-R1, TSLP) was statistically insignificantly decreased in the group of children with asthma (**Figure 1**).

## Usefulness of the Inflammation Markers in the Differentiation of Childhood Asthma

As statistically significant values were obtained only for the MMP-1 marker, its discriminant ability was additionally determined by calculating the ROC curve. A graphical summary of the sensitivity and specificity of the inflammatory marker MMP-1 is presented in **Figure 2**, while the numerical data is shown in **Table 4**. We found the value of the area under the curve close to 0.7 as a satisfactory discriminant factor. The results obtained

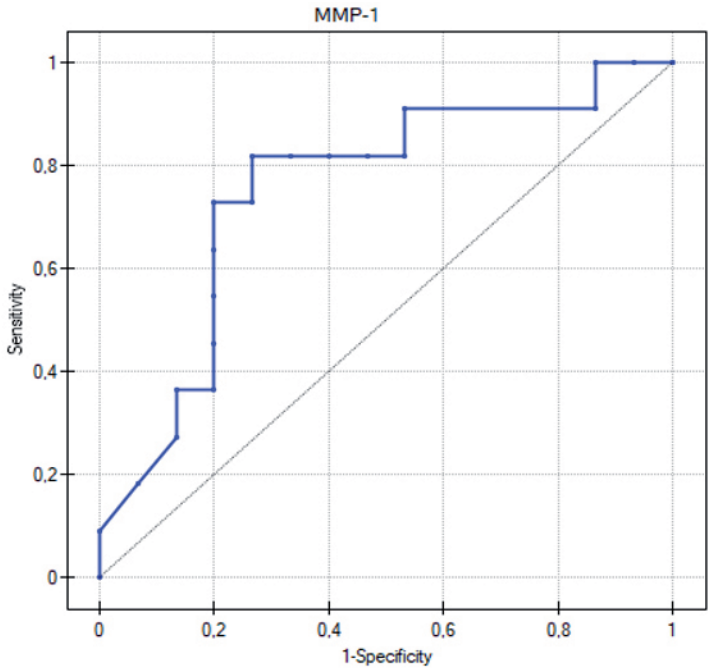


**Figure 2.** Univariate receiver operating characteristic (ROC) curve presents the relationship between the concentration of MMP-1 in the serum in patients with childhood asthma and the participants from the control group

**Table 4.** The discriminant value of expression in serum of the marker MMP-1 showing a significant "p" value and the area under the receiver operating characteristic (ROC) curve (AUC) between the two study groups (with childhood asthma vs. without asthma)

| ROC curve analysis | Inflammatory Factor |
|---|---|
| | MMP-1 |
| AUC | 0,752 |
| SE(AUC) | 0,103 |
| -95%CI | 0,549 |
| +95% CI | 0,954 |
| p value | 0,0313 |

in this study allow to classify the MMP-1 protein as a satisfactory discriminant factor which allows to distinguish childhood asthma from children without asthma and allergies. The AUC (area under the curve) result for the MMP-1 protein (0.752) indicated 73.3% specificity and 81.8% sensitivity with a cut-off of 1160.83 pg/ml for this marker. The differences in the concentrations of other inflammatory proteins between the groups were statistically insignificant, therefore they were not analyzed for the ROC curves.

## Discussion

In view of the high prevalence of asthma among children and the need for prompt treatment, it is important to have measurable indicators that correlate with the development of the disease. The aim of this research was to characterize the cytokine profile of children suffers from asthma using Bio-Plex system. The Bio-Plex assay is similar to the ELISA, both methods detect proteins and use antibodies for this purpose. The Bio-Plex system utilizes xMAP ( the multiplex analyte profiling) technology to enable the multiplexing of up to 100 different analytes [13]. Multiplexed analysis has the advantage of simultaneously detecting multiple analytes in a single reaction vessel reducing time, labor, and cost when compared to single-reaction-based detection methods. For example, the determination of the concentration of 37 inflammatory cytokines with the Bio-Plex method takes only 3 hours, while the ELISA test for the same number of samples takes more than 60 hours. In addition, we used one 96-well plate in this study, an ELISA test would need 37 such reaction vessels. It should also be noted that there is a significant difference in the amount of biological material needed to perform the analysis. The Bio-Plex test requires a maximum of 12.5 µl of serum or plasma, and the ELISA test requires a minimum of 1 ml. Furthermore, the Luminex/xMAP system offers high-throughput detection of target proteins. Due to these properties, the Bio-Plex is an increasingly used method. Previously, the Bio-Plex system was used in research on mucopolysaccharidosis IVA, respiratory and reproductive pathogens in swine, colorectal cancer, achalasia, rhinosinusitis, nephropathy and many other diseases [14–19].

We successfully measured the concentrations of 19 inflammatory factors from the TNF superfamily proteins, IFN family proteins, Treg cytokines, and MMPs (APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, sIL-6Rα, IL-19, IL-26, MMP-1, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP and TWEAK/TNFSF12). According to our results, the serum concentrations of APRIL/TNFSF13, sCD163, gp130/sIL-6Rbeta, sIL-6Ralfa, IL-19, MMP-2, MMP-3, Osteocalcin, sTNF-R2, TWEAK/TNFSF12 were increased in asthmatic group, when compared to healthy participants. In turn, the circulating levels of BAFF/TNFSF13B, sCD30/TNFRSF8, Chitinase 3-like 1, IL-26, Osteopontin (OPN), Pentraxin-3, sTNF-R1, TSLP, were decreased in children suffering from asthma comparing to control group. Although, a statistically significant difference was only obtained for MMP-1 ($p < 0.05$). Based on the univariate ROC curve, that graphically presents the sensitivity and specificity of the analyzed compound, MMP-1 may be considered a prognostic factor for childhood asthma.

Matrix metalloproteinases (MMPs) belong to the family of endopeptidases, which are zinc

dependent and involved in the breakdown of a wide variety of extracellular components. MMPs play a role in physiological as well as pathological processes of structural remodeling including cell migration, tissue repair and tumor necrosis. Interestingly, the expression process of MMP genes is tightly controlled by various cytokines that can activate (e.g. TNF-alpha, IL-1, TGF) or inhibit transcription (e.g. IL-4) [20]. MMPs are synthesized and released in allergic diseases such as asthma by several types of cells, including leukocytes and macrophages, and cells constituting the structure of the airways, such as epithelial cells, fibroblasts and smooth muscle cells [21,22]. Matrix metalloproteinase 1 is responsible for the degradation of the most abundant proteins in the human body – collagen types I, II, III, V, IX and X [21]. For this reason, MMP-1 is essential for the modeling and remodeling of the extracellular matrix [21]. Under physiological conditions, a low level of this protein is noticeable, but the level increases in pathological conditions. Elevated levels of MMP-1 level have been detected in many types of tumors, implant failure, occlusive peripheral arterial, coronary artery disease, osteoporosis and gingivitis or periodontitis [21,23,24]. In this study the concentration of MMP-1 in the serum of children suffering from asthma was reduced when compared to healthy subjects. Contrary to our results, Rogers et al. and Naveed et al. detected increased levels of MMP-1 in adult asthma patients [25,26]. However, to our knowledge, this study for the first time shows this phenomenon in childhood asthma. All differences in the expression of inflammatory factors and mediators of immunity between childhood and adult asthma may be due to the immaturity of the children's immune system [27,28]. Moreover, these scientists did not use the Bio-Plex in their research. The Bio-Plex system was only used by Sugai et al. to study cytokines in childhood asthma, but MMP-1 levels were not measured [29]. Previously, we detected the serum concentration of MMP-1 and MMP-3 in patients suffering from Hymenoptera venom allergy, but we did not observe significant differences between studied groups [12]. Dahlen et al. showed that MMP-3 is present in both mast cells and eosinophils, which are key effector of the asthmatic inflammatory response [30]. Furthermore, MMP-3 has been reported in vitro to be involved in the bioprocessing of pro-TNF alpha. Since TNF alpha is localized in human mast cells, this creates the possibility for MMP-3 to act potentially as an activator of TNF alpha release, thereby enhancing the profibrotic course and influencing endothelial cell activation and the recruitment of infiltrating leucocytes in asthma [31]. There is evidence that MMP-1 may also be involved in the pathogenesis of asthma. According to Ingram et al. the concentration of MMP-1 is related to the IL-13 activity [32]. They observed that IL-13 stimulates the secretion of matrix metalloproteinase 1 [31,32]. Their research also showed that MMP-1 leads to the proteolytic cleavage of membrane-tethered heparin-binding epidermal growth factor (HB-EGF) on the cell surface, which is essential for epithelial repair [31,33]. Damage to the bronchial epithelium can lead to many diseases, including childhood asthma. Moreover, Cataldo et al. reported in their studies that patients with asthma had an elevated level of matrix metalloproteinase 1 gene expression [35]. They explain that bronchial remodeling may be related to this condition and may lead to the development of asthma. The authors also point to a relationship between MMP-1 activity and inflammatory processes in the respiratory tract [35]. Ohta et al. showed the connection between the presence of IL-13 and increased secretion of matrix metalloproteinase 1. Additionally, they proved that the presence of IL-13 causes an increase in the expression level of MMP-1 mRNA and pointed that IL-4 has the same effect as IL-13 [36]. Unfortunately, in our study, we did not measure the levels of IL-4 and IL-13 in children with asthma.

In the development of asthma, a special role is ascribed to immunological processes. In the future, the inhibition/ activation of pro-inflammatory/ anti-inflammatory factors may be helpful in treating children with asthma. Matrix metalloproteinases can be considered as potential therapeutic target. Scientists are eager to look for agents that inhibit overexpression or block the action of the MMP-1 protein. Kim et al. showed that treatment with scopoletin decreases MMP-1 expression by reducing p38 MAPK phosphorylation, which can be used to combat inflammation in keratinocytes [37]. A study conducted on the mouse chondrogenic cell line ATDC5 by Liu et al. identified soya-cerebroside as a potential therapeutic agent for the treatment of osteoar-

thritis due to its properties that inhibit MMP-1 expression [38]. Additionally, the inhibitory effect of triflorethol-A on MMP-1 is investigated in the context of aging processes in human keratinocytes [39]. However, the studies mentioned above require further verification to confirm the effective and purposeful use of these substances in the indicated disease entities. The inhibition of MMP-1 synthesis and secretion should also be investigated as a potential asthma treatment to prevent bronchial remodeling.

In this study, we observed alterations in the serum profile of the remaining 18 inflammatory factors (APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, sIL-6Rα, IL-19, IL-26, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP and TWEAK/TNFSF12) in asthmatic patients, however, the obtained differences were not statistically significant. According to literature, most of these inflammatory factors may be involved in the course of asthma [33]. TNF-R1 and sCD30/TNFRSF8 belong to the tumor necrosis factor receptor (TNFR) superfamily [40]. An elevated level of sCD30 was observed in atopic dermatitis, and it was recognized as a potential marker of clinical severity in this condition. The study proved that sCD30 level was statistically higher in the exacerbation and remission phase of the disease compared to the control group. It was also observed a statistically significant positive correlation between the observed concentration of soluble CD30 receptor and the clinical status of patients in both periods[41]. There are also reports that sCD30 level is increased in allergic asthma [42]. In turn, Boonpiyathad et al. investigated the role of IL-2 in asthma and concluded that the level of IL-2 is higher in patients with severe asthma than in patients with mild asthma [43]. Raundhal et al. report that IL-17 secretion is inhibited, but INF-γ secretion is stimulated in asthma [44]. Charrad et al. revealed increased level of IL-8 and Kim et al. elevated level of TWEAK [44,45]. Our study had some limitations. The lack of statistical significance in our study may be due to the insufficient number of patients due to the pilot nature of the study. Future research should expand the patient population for more reliable results on childhood asthma. Another limitation of our study was the high heterogeneity of the study group in terms of the severity of asthma, comorbidities, and therapy. The obtained results are of a cognitive nature and broaden the understanding of the role of cytokines in the pathogenesis of childhood asthma, however currently they cannot be useful in clinical practice.

## Conclusion

In conclusion, our study showed that the inflammatory protein MMP-1 is an important marker that can be used to recognize childhood asthma endotypes. As chronic inflammation of the airways is crucial for the development of asthma, monitoring the profile of its components should be included in the routine course of patient management. Treatment of asthma is aimed at gaining clinical control and minimizing the risk for the patient in the future. Continuous monitoring is essential to achieve this goal in childhood asthma. Cytokines carry a burden of drivers of immune responses and are therefore key coordinators and fixers of airway inflammation. This makes them an attractive target for treatment but may be at the fore in the diagnosis of asthma in the near future. This topic requires in-depth research to discover as many of the predisposing factors as possible to the development of asthma. Perhaps some immune factors change their levels much earlier than biochemical factors. This state of knowledge would allow for a much faster diagnosis and implementation of appropriate treatment, which is very important in the case of childhood asthma. Future research should also indicate to which endotype matrix metalloproteinase 1 is classified. Due to this fact, this study is only the beginning of more complex proteomic analyzes.


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**Conflict of interest statement**
The authors declare no conflict of interest.

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**Institutional Review Board Statement**
The study was approved by the Local Ethical Committee of the Medical University of Poznan, Poland (Decision No. 530/12), in accordance with the requirements of the Helsinki Declaration.

**Informed Consent Statement**
Informed consent was obtained from the guardians of all children involved in the study.

**Data Availability Statement**
The data presented in this study are contained within the article.

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