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# The usefulness of flow cytometric analysis of cytokines in peripheral blood and bone marrow plasma

## Przydatność oznaczania cytokin w osoczu krwi i szpiku kostnego metodą cytometryczną

### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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### Summary

#### Introduction:

Recently attention has been paid to the role of cytokines in clinical pathology, since they can mediate a wide variety of biological effects. For these reasons multiplex methods have been developed to simultaneously measure numerous cytokines in individual small volume specimens. The aim of the study was to assess the usefulness of flow cytometric analysis of cytokines in peripheral blood and bone marrow plasma with Cytometric Bead Array (CBA) kits.

#### Material and Methods:

The study involved 59 children. Tests were performed in peripheral blood and bone marrow plasma. Human Inflammatory Cytokine Kit (IL-8, -1 $\beta$ , -6, -10, TNF, -12p70) and Human Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>17</sub> Cytokine Kit (IL-2, -4, -6, -10, TNF, INF- $\gamma$ , -17A) (BD Bioscience) were used. Samples were analyzed on a Cytomics FC500 flow cytometer (Beckman Coulter).

#### Results:

In patients diagnosed for hemophagocytic lymphohistiocytosis (HLH) (n=10) and for acute lymphoblastic leukemia (ALL) (n=12) Human Inflammatory Cytokine Kit was used. In almost all samples individual cytokines were detected in a wide range of concentrations (0.47 - 653.74 pg/ml). In samples from patients suffering from allergy (n=12) and in healthy children (n=25) Human Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>17</sub> Cytokine Kit was used. Detection of individual cytokines was much lower: concentration range 0.09-30.17 pg/ml.

#### Discussion:

Based on our analysis the CBA test is suitable for analysis of several cytokines in small volumes of samples. A simple flow cytometer can be used for this test. The CBA test is more suitable for samples with expected increased levels of cytokines. When the levels of cytokines are low, the sensitivity of the CBA test can be too low.

#### Keywords:

CBA • children • cytokines • allergy • HLH • ALL

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## INTRODUCTION

Cytokines regulate important biological processes such as the immune response or hematopoiesis and are involved in pathogenesis of many diseases. In the physiological state their concentration in biological fluids and tissues is undetectable or very low. Therefore, any increase in their concentration suggests activation of pathways involved in an inflammatory response or disease development. That is why cytokines may serve as potential biomarkers of various diseases, and changes of their concentration may be used in follow-up. Moreover, the cytokine profile in the initial (acute) phase of the disease often differs from the chronic phase profile [23].

Measurement of cytokine concentration is sufficient to diagnose a disease only in a few cases, e.g. in familial hemophagocytic lymphohistiocytosis (HLH) increased concentration of soluble interleukin 2 receptor in serum is observed. The concentration and profile of cytokines correlate with the stage of the disease and might be used to assess prognosis. For example, delayed increase of IL-6 in patients after severe trauma suggests development of multiorgan failure. Measurement of IL-6 and IL-8 level is clinically significant in bacterial sepsis in newborns and hence can be useful in differential diagnosis [9,10,19,23].

The measurement of a single cytokine has limited clinical significance. To diagnose or assess the prognosis a characteristic profile of cytokines obtained by measurement of a larger number of cytokines is needed. The pathogenesis of many diseases is related to imbalance between Th<sub>1</sub> and Th<sub>2</sub> subpopulations. Excessive production of Th<sub>1</sub>-dependent cytokines is observed in cellular response-driven autoimmune diseases, such as rheumatoid arthritis or type 1 diabetes. High levels of Th<sub>1</sub>-dependent cytokines are responsible for graft rejection or recurrent miscarriages. On the other hand, predominance of Th<sub>2</sub>-dependent cytokines is involved in the development of allergic diseases and other diseases driven by autoantibodies (SLE, pemphigus). The excess of these cytokines is related to hypereosinophilia, tumor metastases and AIDS progression. Special interest is given to newly described T-helper cell subpopulations and their role in disease pathogenesis. It was shown that Th<sub>17</sub> cells secreting IL-17 and IL-22 are involved in autoimmune and allergic reactions [1,9].

Simultaneous measurement of a set of cytokines is used to follow up the efficacy of tumor immunotherapy. In addition, the method is used in follow-up of transplant recipients and patients with autoimmune diseases. It can also be useful to assess the immune response after administration of vaccines, e.g. against flu or rubella. Parallel measurement of IL-6/IL-10/TNF- $\alpha$  is clinically significant in early and fast diagnosis of G<sup>+</sup>/G<sup>-</sup> bacteremia in pediatric patients hospitalized in hematology and oncology wards. More and more attention is paid to the role of cytokine level measurements in patients with allergy and the changes of cytokine levels during specific

allergen immunotherapy [20]. Utilization of cytokine measurements in clinical practice requires development of methods allowing measurement of many cytokines at the same time, i.e. assessment of cytokine profile [3,11,21,22]. A gold standard in cytokine level measurements is enzyme-linked immunosorbent assays (ELISA); however, such methods usually only allow one to assess the level of a single cytokine. Recently, many new, refined methods allowing multiparameter analysis of a single biological sample have been developed. Similarly to ELISA assays, the new methods are based on formation of immunological complexes using two monoclonal antibodies.

Currently, three major cytokine measurement systems based on monoclonal antibodies and fluorescent-labeled molecules are available: Cytometric Bead Array (CBA) (BD Bioscience) or FlowCytomix (eBioscience), Luminex xMAP Technology (Luminex) as well as tests developed by Meso Scale Discovery. Different methods utilize various techniques to distinguish each bead population. In CBA and FlowCytomix assays microspheres are labeled using a single dye, while in xMAP technology two different fluorochromes are bound to the bead to increase the number of detectable parameters. However, xMAP analysis requires specialized equipment manufactured by Luminex, while the CBA test can be performed using the majority of cytometers used in routine diagnostics (equipped with blue laser – 488 nm, detection at two wavelengths). The Meso Scale Discovery system is based on electrochemiluminescence [2,6,12,15,18].

The aim of the study was to adjust Cytometric Bead Array to the equipment and conditions of the routine diagnostic laboratory and to assess the usefulness of the method to measure the cytokine profile in two different biological materials: blood plasma and bone marrow plasma, in children in various clinical conditions.

## MATERIALS AND METHODS

The studied group was composed of 59 children, including: 12 children (mean age  $\pm$  SD: 5.5 $\pm$ 3.2) with confirmed tree and cereal pollen inhalation allergy (the tests were performed using peripheral blood plasma), 10 children (mean age  $\pm$  SD: 6.3 $\pm$ 6.6) examined for hemophagocytic lymphohistiocytosis (HLH) (the tests were performed using peripheral blood plasma), 12 children (mean age  $\pm$  SD: 5.5 $\pm$ 3.2) with confirmed acute lymphoblastic leukemia (ALL) at the day of diagnosis (the tests were performed using bone marrow plasma left after diagnostic tests for ALL) and 25 healthy children (mean age  $\pm$  SD: 5.5 $\pm$ 4.2) (the tests were performed using peripheral blood plasma).

The study was retrospective, and the tests were performed using material left after routine tests. Both peripheral blood and bone marrow were drawn into tubes containing EDTA. Immediately after drawing, the samples were centrifuged (5

minutes, 200 g) and plasma was collected promptly. The material was frozen (during 30 minutes after drawing) at -20°C.

Two different kits for measurement of cytokine panels were used: Human Inflammatory Cytokine Kit and Human Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>17</sub> Cytokine Kit (both from BD Bioscience).

Human Inflammatory Cytokine Kit (allowing one to measure the concentration of IL-8, -1β, -6, -10, TNF, and IL-12p70) was used to assess cytokine profile in blood plasma obtained from patients examined for HLH and in bone marrow plasma of children diagnosed with ALL. Human Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>17</sub> Cytokine Kit was used to measure the concentration of IL-2, -4, -6, -10, TNF, INF-γ, and IL-17A in blood plasma obtained from children with inhalation allergy and healthy controls.

The tests were performed according to the manufacturer's protocols. Human Inflammatory Cytokine Kit: 50 μl of assay beads, 50 μl of the studied sample or standard and 50 μl of PE-labeled antibodies (Detection Reagent) were added consecutively to each sample tube. The samples were incubated at room temperature in the dark for 1.5 h. Next, the samples were washed with 1 ml of Wash Buffer, centrifuged and the resulting pellet was resuspended in 50 μl of Detection Reagent. The samples were further incubated for 1.5 h, washed again and centrifuged. After discarding the supernatant, the pellet was resuspended in 300 μl of Wash Buffer and analyzed on the same day in a flow cytometer.

Human Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>17</sub> Cytokine Kit: 50 μl of assay beads, 50 μl of Detection Reagent and 50 μl of the studied sample or standard were added consecutively to each sample tube and incubated for 3 h at room temperature, in the dark. Next, the samples were washed with 1 ml of Wash buffer, and centrifuged. After discarding the supernatant, the pellet was resuspended in 300 μl of buffer and analyzed on the same day in a flow cytometer.

All samples were analyzed using Cytomics FC 500 (Beckman Coulter) device and FCAP Array v2.0 software (Soft Flow, Hungary). Before the analysis the cytometer was calibrated using Set-up Beads according to the manufacturer's protocol.

The cytometric analysis of standards generated 10 cytograms (FL4 vs FL2) for each kit. Each time, cell populations were distinguished on the basis of FL4 fluorescence. Subsequent histograms showed shifting of the observed molecules on the x axis (FL2) caused by increasing concentration of the studied cytokines in the studied standards. An example of dot-plots (cytograms) is shown in Fig. 1

FCAP Array software was used to calculate mean fluorescence intensity (MFI) for each studied population of molecules able to bind to a given cytokine. The obtained results were used to create a graphical representation of the relationship between concentration of a given cytokine and fluorescence intensity and to choose a proper statistical model. As a result, 6 standard curves (4 PL) were obtained and used to assess the concentration of cytokines in the studied samples. An example of a standard curve for IL-10 is shown in Fig. 1.

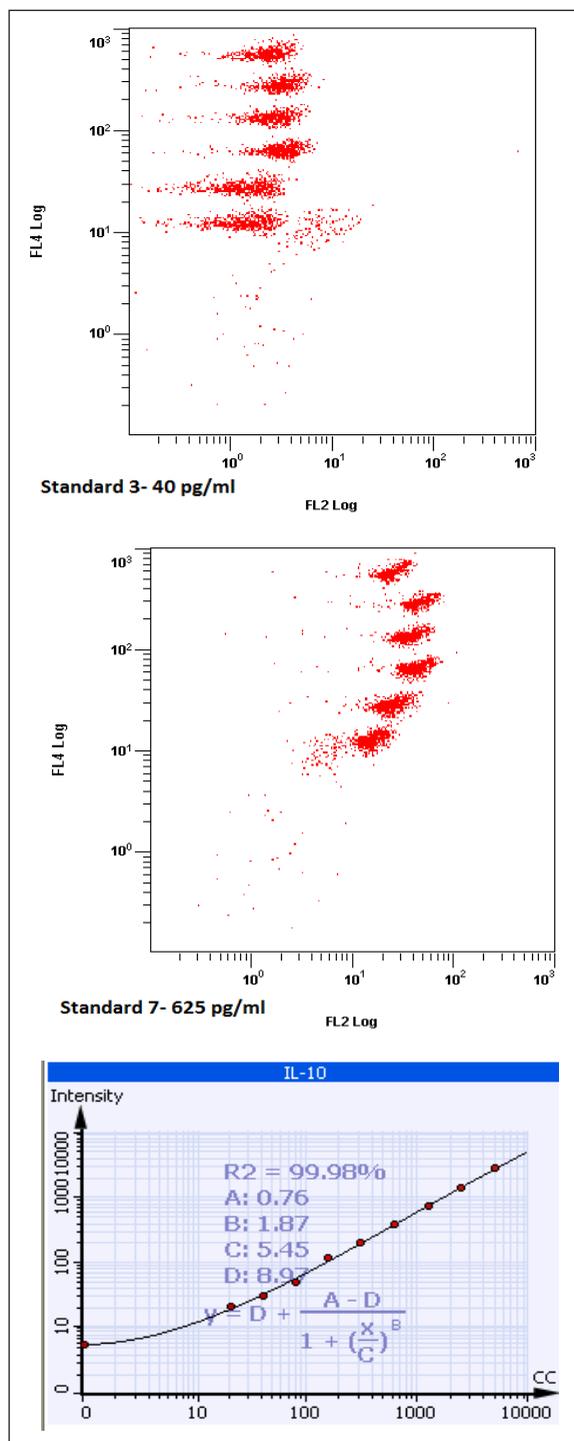


Fig. 1. Cytometric analysis of two standard samples (40 and 625 pg/ml) shown as FL4 vs FL2 cytograms and standard curve for IL-10 generated in FCAP Array software. The curve shows the relationship between concentration (CC) and fluorescence intensity (Intensity). To create the curve in a logarithmic scale a 4-parameter logistic curve was used (4-PL). The graph shows values for respective parameters (A, B, C, D), the equation of the curve and the correlation coefficient (R2)

The shape and adjusting of the standard curve significantly influence the accuracy of the obtained results. The curves used for the presented experiments were characterized by very high (99.98 and 99.99) regression coefficients (in per-

centage), confirming that the regression model used was suitable. Theoretical measurement sensitivity for each of the studied cytokines was provided by the manufacturer as the detection limit value. However, the linear part of the curves obtained during the experiments allowing repetitive interpolation of fluorescence intensity started over 20 pg/ml.

**RESULTS**

**Test Human CBA Inflammatory Cytokine Kit**

*Cytokine measurements in blood plasma obtained from patients examined for HLH*

In 9 patients, all 6 studied cytokines were detected in a broad range of concentrations (Table 1). Only in 1 of 10 analyzed samples were the concentrations of all studied cytokines undetectable. An example of the obtained results of cytometric analysis and representative cytokine profile is shown in Fig. 2.

Table 1. Results of cytokine measurements in blood plasma obtained from children diagnosed for HLH using CBA Inflammatory Kit

Cytokine	Number of samples with detectable level /number of studied samples	Mean ± SD (detection range) (pg/ml)
IL-8	9/10	84.30 ± 19.76 (62.32-116.37)
IL-1 β	9/10	18.20 ± 5.66 (6.29-25.05)
IL-6	9/10	45.12 ± 23.49 (25.75-104.14)
IL-10	9/10	44.49 ± 24.52 (31.97-109.03)
TNF	9/10	38.19 ± 10.97 (12.28-50.35)
IL-12p70	9/10	31.98 ± 10.12 (9.97-44.27)

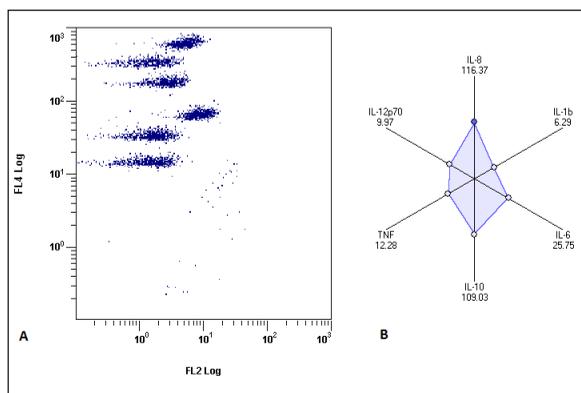


Fig. 2. An exemplary result of cytokine concentration measurement in blood plasma obtained from patients diagnosed for HLH. The FL2 vs FL4 cytogram (A) shows localization of six populations of cytokine binding beads while the Radar Plot (B) shows concentrations of particular cytokines (pg/ml)

*Cytokine measurements in bone marrow plasma obtained from patients diagnosed with ALL*

The results of the measurements performed in bone marrow plasma obtained from ALL patients were heterogeneous (Table 2). IL-8 and IL-1 β were found in all 12 studied samples. In some patients high levels of all studied cytokines were found (Fig. 3)

Table 2. Results of cytokine measurements in blood plasma obtained from children diagnosed with ALL using CBA Inflammatory Kit

Cytokine	Number of samples with detectable level /number of studied samples	Mean ± SD (detection range) (pg/ml)
IL-8	12/12	228.50 ± 209.76 (6.75-615.25)
IL-1b	12/12	112.01 ± 102.91 (2.6-239.22)
IL-6	11/12	127.87 ± 123.19 (0.47-298.59)
IL-10	11/12	106.88 ± 96.55 (0.58-269.07)
TNF	8/12	188.77 ± 173.74 (5.99-420.81)
IL-12p70	8/12	255.83 ± 249.39 (8.06-653.74)

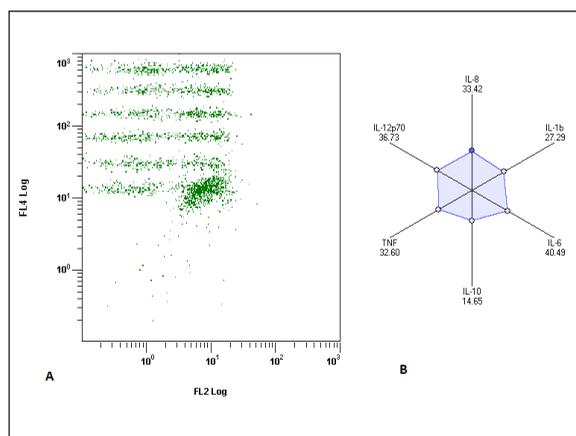


Fig. 3. An exemplary result of cytokine concentration measurement in bone marrow plasma in a patient diagnosed with ALL (CBA Inflammatory Kit). The FL2 vs FL4 cytogram (A) shows localization of six populations of cytokine binding beads while the Radar Plot (B) shows increased concentrations of the studied cytokines (pg/ml)

In other samples the level of a single or a few cytokines was high, whereas the remaining cytokines were undetectable or in low concentrations (Fig. 4).

**CBA Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>17</sub> Cytokine Kit**

*Cytokine measurements in blood plasma obtained from patients with allergy*

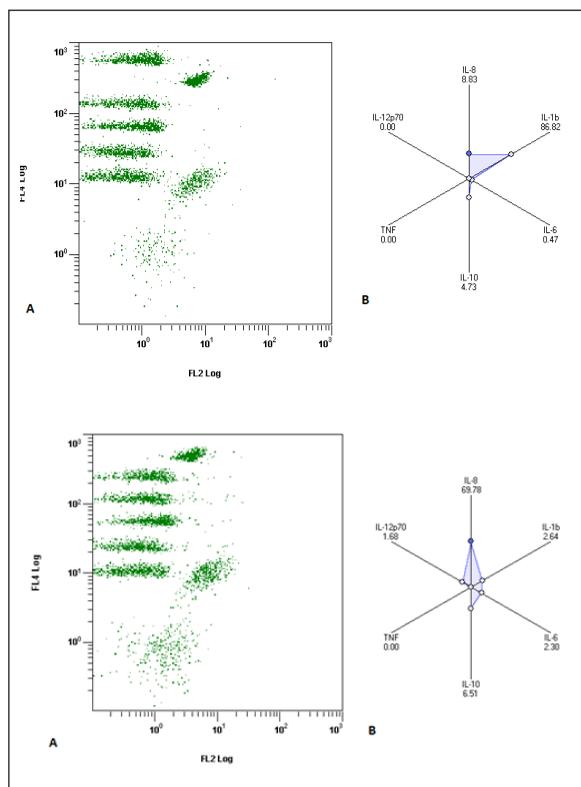


Fig. 4. Exemplary results of cytokine concentration measurement (CBA Inflammatory Kit) in bone marrow plasma of two patients diagnosed with ALL. The FL2 vs FL4 cytogram (A) shows localization of six populations of cytokine binding beads while the Radar Plot (B) shows respective concentrations of the studied cytokines (pg/ml)

Table 3. Results of cytokine measurements in blood plasma obtained from children with allergy using CBA Th1/Th2/Th17 assay

Cytokine	Number of samples with detectable level / number of studied samples	Mean ± SD (detection range) (pg/ml)
IL-2	5/12	0.14 ± 0.22 (0.19-0.72)
IL-4	8/12	0.32 ± 0.37 (0.09-0.97)
IL-6	5/12	0.61 ± 0.59 (0.25-1.12)
IL-10	5/12	0.06 ± 0.22 (0.25-1.12)
TNF	1/12	0.06 ± 0.22 (0.79)
INF γ	3/12	0.26 ± 0.54 (0.4-1.61)
IL-17A	3/12	0.14 ± 0.22 (0.19-0.72)

The results of cytokine concentration measurements in samples obtained from 12 children with inhalation allergy are summarized in Table 3.

The majority of the studied cytokines were not detected in allergic patients' plasma or the obtained

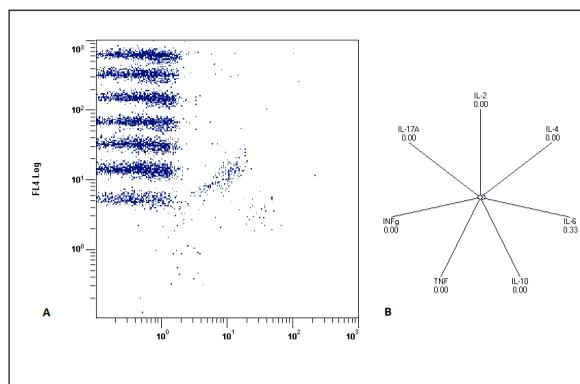


Fig. 5. An exemplary result of cytokine concentration measurement (CBA Th1/Th2/Th17) in blood plasma obtained from a patient with allergy and undetectable or very low concentration of the cytokines. The FL2 vs FL4 cytogram (A) shows localization of seven populations of cytokine binding beads while the Radar Plot (B) shows concentrations of the studied cytokines (pg/ml)

results suggested very low concentration (below 1 pg/ml). An example of such a result is shown in Fig. 5.

In three patients the IL-17 A level was strongly increased in comparison to other cytokines measured (Fig. 6).

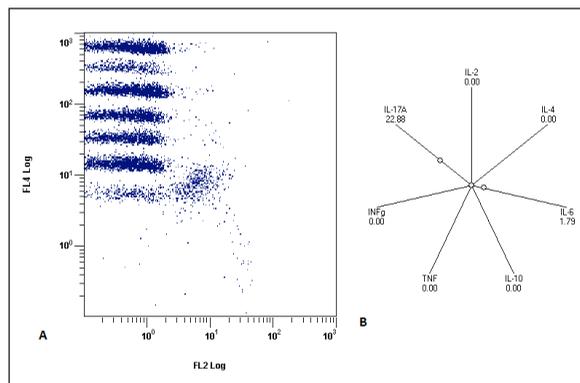


Fig. 6. An exemplary result of cytokine concentration measurement (Th1/Th2/Th17) in blood plasma obtained from a patient with allergy and increased concentration of IL-17A. The FL2 vs FL4 cytogram (A) shows localization of seven populations of cytokine binding beads while the Radar Plot (B) shows concentrations of the studied cytokines (pg/ml)

According to the manufacturer protocol suggestions, blood plasma of a few subjects with undetectable level of cytokines was diluted before re-testing. However, subsequent analysis did not change the obtained results.

*Cytokine measurements in blood plasma obtained from healthy children*

Similarly to allergic patients, concentrations of the studied cytokines in the majority of healthy patients were low or undetectable (Fig. 7).

The only exception was IL-17A concentration, which was detectable in a few subjects (4.65-30.17 pg/ml). The results are summarized in Table 4.

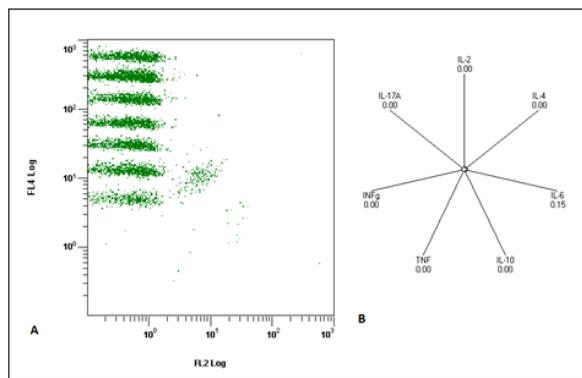


Fig. 7. Exemplary results of cytokine concentration measurement (CBA Th1/Th2/Th17) in blood plasma obtained from a healthy child with undetectable or very low concentration of the cytokines. The FL2 vs FL4 cytogram (A) shows localization of seven populations of cytokine binding beads while the Radar Plot (B) shows concentrations of the studied cytokines (pg/ml)

Table 4. Results of cytokine measurements in blood plasma obtained from healthy children using CBA Th1/Th2/Th17 assay

Cytokine	Number of samples with detectable level / number of studied samples	Mean ± SD (detection range) (pg/ml)
IL-2	9/25	0.55 ± 1.29 (0.25-2.6)
IL-4	10/25	0.74 ± 1.54 (0.25-2.17)
IL-6	14/25	2.36 ± 8.96 (0.25-2.24)
IL-10	18/25	1.02 ± 1.38 (0.25-2.6)
TNF	9/25	0.97 ± 2.23 (0.4-4.21)
INF γ	7/25	1.19 ± 2.92 (0.12-3.19)
IL-17A	9/25	10.85 ± 19.66 (4.65-30.17)

## DISCUSSION

Increasing data on cytokines and their modulatory role in various biological processes (both physiological and pathological) caused growing interest in the use of cytokine level measurements in various biological samples. Our study confirmed that Cytometric Bead Array is useful in measurements of cytokine concentration in blood and bone marrow plasma. Recently published literature has shown that the CBA system might be used to measure cytokine concentrations in various biological fluids, including tears or expired air condensate [5]. However, the most attention is paid to the assessment of systemic concentration of cytokines using blood plasma and serum. Published data suggest that these two materials are not equivalent. Because of that, more commonly the tests are performed on blood plasma to avoid the influence of blood clotting on the level of cytokines [7]. This is why in the presented study blood plasma was used instead of serum.

The studied material was obtained from pediatric patients in whom only a limited amount of blood can be drawn for analyses. CBA methods allow one to use a small amount of blood, as only 50 µl of the studied material is sufficient to perform the test. Moreover, 6-7 or even more cytokine concentrations can be measured in such a small amount of sample. Therefore, as emphasized in previously published studies, the CBA method seems to be particularly useful to measure cytokine levels in children [19,22]. Moreover, the CBA method is easy to perform and allows one to obtain multiparametric results in a relatively short time (4-5 hours). Appropriate calibration and adjustment of the cytometer for CBA tests is easy and does not require additional qualifications apart from basic knowledge on using a flow cytometer.

Because of the use of fluorescent labeled monoclonal antibodies, Cytometric Bead Array is relatively expensive; however, the main advantage of this method is that it can be performed on any flow cytometer used in routine diagnostics (equipped with at least one 488 nm laser). Therefore, CBA does not require any additional equipment, which reduces the costs in comparison to other multiplex methods such as Luminex or Meso [5,6,11,12].

On the other hand, the cost of the test is increased by the need for preparation of a 10-point standard curve (manufacturer's recommendations) together with each set of studied samples. The studies performed by Verdine et al. [25] have shown that diluted standards cannot be used again, because cytokine concentrations change during storage. Moreover, the authors have shown that a standard curve performed using freshly diluted standards is stable for a few weeks. Therefore, it is possible to analyze the standard curve periodically (e.g. once a week), and this approach was applied in our study.

According to the recent publications, another advantage of the CBA method is the very broad range of tested concentrations (20-5000 pg/ml) provided by appropriate design of the standard curve [22]. Cytokine concentrations exceeding 5000 pg/ml are very rarely observed both in physiological and pathological conditions. In our material none of the studied samples exceeded that level and there was no need to dilute the samples. Hence, the CBA method saves time and reduces the cost of sample dilutions and re-testing.

The studies performed in various groups of patients showed that CBA is particularly useful to measure cytokine concentrations in conditions related to significant hypercytokinemia. The majority of the studied cytokines (CBA Inflammatory Kit) were detected in patients with HLH or leukemia. Concentrations of the studied cytokines broadly varied (up to 653.74 pg/ml). Suitability of the CBA test to measure high cytokine concentrations in pediatric patients was confirmed in other publications [23,24].

However, in clinical conditions characterized by lower concentrations of cytokines the possibility to detect cytokines using the CBA test is limited. In the studied group of pa-

tients with allergy the majority of the measured cytokines were undetectable or the concentrations were very low. Similar results were obtained for healthy children, corresponding to very low peripheral concentrations of these cytokines in physiological conditions with increased concentrations locally [17]. In the case of children with allergy we expected increased concentrations of some cytokines, e.g. those related to Th<sub>2</sub> [1].

There are a few reasons why the cytokines were not detected using CBA. Detection problems might be due to biological characteristics of the studied cytokines, especially their short half-life [8]. To protect the material from cytokine degradation, blood plasma was separated and stored at -20°C just after the sample was drawn. The samples were stored for a short period of time (at least a few months) and therefore there was no need to store them at a lower temperature. The same method of storage was used for samples in which high concentrations of cytokines were found. Some authors claim that the presence of carrier proteins or heterophile antibodies in the studied material might be the cause of false negative results [9, 25]. However, it is observed usually in patients in poor general condition and is not the case in children with inhalation allergy. According to the manufacturer's guidelines, such interference can be limited by sample dilution. However, additional analysis of the diluted samples did not change the obtained results significantly (data not shown).

One of the most commonly underlined causes of detection problems is inadequate sensitivity of CBA [7, 21]. The latest studies showed that theoretical detection limits given by the manufacturer do not always reflect the real sensitivity of the test [4]. Low sensitivity is caused mainly by the design of the standard curve, allowing a very broad assessment range (up to 5000 pg/ml) at the cost of the number of low-concentration standards. Extrapolation of the results in lower ranges might not be sensitive enough, and some authors suggest that in such cases the result should be analyzed as "below the lowest standard" (20 pg/ml) instead of the extrapolated concentration [15]. The latest publications show that modification of standard curves by the addition of standard dilutions or use of a new, more sensitive CBA method (Enhanced Sensitivity Flex Set) can solve this problem [6, 16]. Additional low-concentration standards were added to the standard curve of CBA Flex Set (0.274, 0.823, 7.4 pg/ml), and therefore it seems to be more suitable for conditions with a less prominent increase in cytokine concentration (e.g. allergy).

The possibility to assess many parameters in a single sample using Cytometric Bead Array encouraged the broad use of this system in biology and medicine. It is particularly useful in measurement of soluble mediators, including cytokines, related to regulation of the immune system. A few kits to measure particular cytokine profiles in humans are available. These tests may be used to assess ongoing immunological processes characterized by overproduction of certain cytokines. It was shown that CBA can be useful in diagnostics and follow-up. Increased concentration of

pro-inflammatory cytokines, such as IL-6, -8, -10 (measured using CBA Human Inflammatory Kit) in serum or blood plasma might be used in early diagnostics of sepsis both in newborns and adults [13, 25]. In the case of newborns such a panel might be useful in determination of the moment of infection (before or after birth). The same cytokine panel was used to follow up pediatric patients undergoing implantation of heart-lung bypasses [12]. The study showed time-dependent changes of cytokine concentrations in serum: increased concentrations in the perioperative stage and decrease in the healing period. In addition, the CBA method is also used to assess concentrations of cytokines in materials other than serum and blood plasma, such as nasal discharge or sputum. Moreover, the cytokine profile might be assessed even in small samples of tears (5-10 µl), which can be useful in allergy diagnosis [5].

In the majority of studies mentioned above, cytokine concentrations were simultaneously measured using ELISA. The results of multiplex assays correlate well with the results of the gold standard method (ELISA). However, respective concentration values may vary because of different antibodies used to capture and recognize the cytokines. The CBA method has a broader dynamic range of measurement in comparison to ELISA and because of that sample dilutions are rarely needed. In some cases, CBA allows one to detect cytokines in samples undetectable using ELISA. It was shown for cytokine measurements in urine samples obtained from patients after kidney transplant [14].

Although the popularity of multiplex immune methods (including the CBA system) is increasing, they still remain relatively uncommonly used (and less standardized) in comparison to ELISA. There is a need to perform further studies on their suitability to assess cytokine concentration in clinical settings. Unfortunately, the results of CBA tests can be affected by various factors, including biological features of cytokines, appropriate sample preparation as well as the presence of interfering substances. These adverse influences of external factors are observed also in other immunological methods; however, the likelihood of interference increases with the number of parameters measured simultaneously. Such interference should be considered both when performing the test and during interpretation of the obtained result [12, 15].

Our results confirmed that CBA is useful to assess the cytokine profile in small volume samples of biological fluids such as blood and bone marrow plasma. It allows one to freely choose cytokines to be measured. The test can be performed on cytometers used in routine diagnostics and the adaptation of the method to the equipment used is easy. CBA is particularly useful to measure high concentrations of cytokines. In clinical conditions with a less prominent increase in cytokine concentration (in our material – healthy controls and allergic patients) sensitivity of the method is insufficient.

To conclude, CBA tests can be considered competitive to the routinely used immunoenzymatic assays.

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