

Role of pro- and anti-inflammatory cytokines in activation of inflammation at community-acquired pneumonia of children with different level of physical development

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Abstract

Background: With the aim of better predicting the course of the community-acquired uncomplicated pneumonia (CAUP) the study presents the results of research of the cytokine profile based on the obtained rates of pro- (interleukin 1-beta [IL-1 β], tumor necrosis factor-alpha [TNF- α]) and anti-inflammatory (interleukin 4 [IL-4]) cytokines in blood serum as well as results of the combined bacteriological and serological research of children with different level of physical development (PD), sick on CAUP.

Methods: In order to reach the purpose of the research the work was carried out by controlled computer-generated randomization schedule in 2 stages. At the 1st stage the examination of 151 children with CAUP aged from 3 to 14 years from the onset of the disease and of 20 apparently healthy children (control group) was held. All children passed a study of sputum using staining of swabs according to Gram, culture test and determination of causative agents of pneumonia by the method of enzyme multiplied immunoassay (ELISA); in addition, the cytokine profile (IL-1 β , IL-4 and TNF- α) was determined by the method of enzyme-linked immunosorbent assay. At the 2nd stage, according to the inclusion/exclusion criteria, 151 children in the dynamics of the disease were examined: on 7.2 ± 0.9 day from the onset of the disease, the cytokine profile rates were evaluated, and on 12.6 ± 0.9 day the level of IgM, IgG to atypical causative agents of pneumonia were recorded.

Results: The results obtained during the research of the indices of the pro-inflammatory (IL-1 β and TNF- α) and anti-inflammatory (IL-4) cytokines in blood serum of the children with CAUP and having different level of PD can be considered as factors for individually predicting the course of the CAUP of children with different PD.

Conclusion: Data suggest that the ratio of pro- and anti-inflammatory cytokines during inflammatory process in children with CAUP with different PD can be one of the additional, but not unique, factors for predicting the course of the disease.

Keywords

Community-acquired pneumonia, cytokines, children, physical development.

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Introduction

At present, in many countries of the world, community-acquired pneumonia (CAP) takes one of the leading places in the structure of the total sick rate and mortality of children [1, 2], preeminently due to difficult course [3], and frequent complications and features of a premorbid background [2, 4, 5]. That is why the issue of forecasting of the course, possible complications formation [5, 6], increasing of the treatment effectiveness [4, 7, 8] and prevention of CAP of children remain urgent [9, 10].

In recent years, there is growing evidence in international literature about the important role of cytokines as disease marker in the pathogenesis of respiratory diseases [9, 11-13]. However, the peculiarities of the imbalance of cytokines (interleukin 1-beta [IL-1 β], interleukin 4 [IL-4] and tumor necrosis factor-alpha [TNF- α]) on the treatment background are not a sufficiently studied issue in pediatrics. There is no consensus on which of the cytokines play a major pathogenetic role in the development of pneumonia in children [2, 13, 14]. The question of immune response of the organism of a child who has a deviation in the indices of physical development (PD) [15, 16] in one way

or another to community-acquired uncomplicated pneumonia (CAUP) is not still clarified [2, 14, 17]. It is believed that one of the risk factors for developing children pneumonia is low weight [1, 6], but this still leaves an open issue concerning features of the imbalance of cytokines of such patients [18].

All of the above-mentioned caused the relevance of this research, the purpose of which is to study the peculiarities of cytokine profile in children with different PD level sick on CAUP on the grounds of the research of the rates of pro-(IL-1 β and TNF- α) and anti-inflammatory (IL-4) cytokines in blood serum.

Materials and methods

In order to reach the purpose of the research, the work was carried out by controlled computer-generated randomization schedule in 2 stages. At the first stage, by random sampling, 151 children with CAUP aged from 3 to 14 years were examined on 2.2 ± 0.8 day from the onset of the disease when they were admitted to Pediatric Department of Municipal Multidisciplinary Clinical Hospital of the City of Kharkiv, Ukraine. The verification of the diagnosis took place on the grounds of generally accepted methods according to the protocols of diagnostics and treatment [7, 19]. The evidence for the hospitalization of patients was the presence of signs of intoxication, fever higher than 38.5°C and a decrease in the saturation level [7, 10]. The randomization of patients into groups was carried out with the formation of intervention groups.

At this stage, the main three treatment groups were formed: the I group – 50 children (33.11%) with overweight (OWT), the II group – 50 children (33.11%) with body weight deficiency (BWD), the III group – 51 (33.78%) children with average physical development indices (APDI). According to the design of the research, the selection of patients was carried out in accordance with “inclusion/exclusion” criteria. Inclusion criterion: patients aged from 3 to 14 years with CAUP and different level of PD; 2-3 days from the onset of the disease; uncomplicated course of the disease; the focal nature of the inflammatory process in the lungs. Exclusion criteria: refusal of parents to take part in the research; endocrine obesity; receiving antibiotic therapy before admission to hospital; confirmed viral etiology of pneumonia at the moment of hospitalization; availability of vaccine

data against *H. influenzae type b* and pneumococcal infections [10, 11, 20, 21].

The random sampling of children from 3 to 14 years old who formed control group was made at random concurrently with the formation of main groups. The parents gave the written consent for participation of the child in the research. The randomization of the control group was held using the software which generated a random sequence. Afterwards the samples were collected. The control group consists of 20 (11.69%) apparently healthy children of the corresponding age, selected according to the following criteria: absence of chronic somatic diseases; absence of acute diseases during 3 weeks before the examination; absence of complaints; compliance of passport age with biological.

There was also a distribution of children with CAUP by age; the younger group: children aged from 3 to 7 years old ($n = 80$); the older age group: children aged from 8 to 14 years old ($n = 71$). The distribution of children of the main groups by gender and age is represented in **Tab. 1**.

At the 1st stage of the research, the complaints, the history of the disease and life of the children were studied in depth the clinical examination was implemented with an assessment of the level of PD. The examination included general clinical blood and urine tests, bacteriological and serological studies for the purpose of determination of the CAUP causative agent, determination of the cytokine profile in blood serum (IL-1 β , IL-4 and TNF- α) and chest X-ray examination.

After receiving rates of height and body weight, the children PD level was evaluated: from 3 to 5 years – according to the nomograms recommended by WHO and Orders of the Ministry of Health of Ukraine [22]; from 6 to 14 years – according to the Order of the Ministry of Health of Ukraine [23]; the assessment of the body mass index (BMI) was

carried out according to the formula $BMI = m/h^2$, where m is body weight in kg, and h is height in m.

The standard method of bacterioscopy of sputum smears painted on Gram and serological study of antibodies to atypical pathogens of pneumonia were used [20, 24, 25]. The material for bacteriological examination was sputum obtained during coughing up to the start of antibacterial therapy in 121 (80.13%) children with CAUP. The bacteriological study did not include 30 (19.87%) patients due to the refusal of their parents from the examination, because of the absence of productive cough and impossibility of spitting. The results of sputum study in children with CAUP were considered to be diagnostically significant only in the case of detection of a pathogen in titre of not less than 10^6 colonies of the forming units in 1 ml. The serological study of antibodies level (IgM, IgG) to atypical pathogens of pneumonia was conducted by enzyme multiplied immunoassay (ELISA) using test systems (“Savyon”, Israel; “Best”, Russia) for quantitative and semi-quantitative analysis [25]. The results were considered to be positive in the presence of the diagnostic titre of IgM and the probable growth of IgG in paired serum samples.

The determination of the cytokine profile in blood serum was carried out using the enzyme-linked immunosorbent assay on the unit “BioTek” Elx800 (USA, 2012) and standard reagent sets: “IFA-TNF-ALPHA”, “IFA-IL-4”, “IFA-IL-1 β ” (Cytokine LLC, Russia).

The examination plan at the 2nd stage of the research included the study of data on the dynamics of the disease in 151 children, namely, clinical and laboratory examinations: general clinical blood and urine tests, blood sampling for examination of the cytokines level was held on 7.2 ± 0.9 day from the onset of the disease, and the determination of specific IgM, IgG on 12.6 ± 0.9 day; X-ray of the chest was performed in the absence of the

Table 1. The distribution of children with community-acquired uncomplicated pneumonia (CAUP) by gender and age.

Age, years	Younger group, n = 80		Age, years	Older group, n = 71	
	girls	boys		girls	boys
3	9 (5.96%)	7 (4.64%)	8	5 (3.31%)	5 (3.31%)
4	8 (5.29%)	7 (4.64%)	9	5 (3.31%)	6 (3.97%)
5	10 (6.62%)	7 (4.64%)	10	5 (3.31%)	5 (3.31%)
6	9 (5.96%)	6 (3.97%)	11	5 (3.31%)	5 (3.31%)
7	10 (6.62%)	7 (4.64%)	12	5 (3.31%)	5 (3.31%)
Total	46 (30.46%)	34 (22.52%)	13	5 (3.31%)	5 (3.31%)
			14	5 (3.31%)	5 (3.31%)
			Total	35 (23.18%)	36 (23.84%)

positive dynamics. The obtained results were compared with the data of apparently healthy children. Serologic examination was performed for 65 (43.05%) children by identifying IgM and IgG to causative agents of pneumonia in paired serum: 28 (18.55%) children were serologically examined; 37 (24.5%) patients had combined examination: serological and bacteriological; 86 (56.9%) children were not included in the study in condition of the “exclusion” and/or in the absence of productive cough and impossibility of spitting of the child.

The results analysis was carried out using software “Excel® for Windows®” and “STATISTICA 6.0 for Windows®” by the methods of variation statistics with the standard software package SPSS® for Windows® 9.0 [26].

The research was made in accordance with the requirements of the European Convention on the Protection of Human Rights and Dignity in the Biomedicine and the relevant laws of Ukraine and the principles of the Helsinki Declaration of Human Rights, with minimal psychological loss on the part of patients, with the written consent of the parents of patients for non-harmful research of their children and with confidentiality concerning personal information of the patient.

Results

The examination results analysis showed that the CAUP clinical course has certain features depending on the children PD level. Thus, among the general (weakness, adynamia, loss of appetite, fever) and respiratory (cough, sputum secretion, shortness of breath) symptoms, the greatest manifestations were observed among the I and II groups ($20.1\% \pm 1.7\%$ and $19.2\% \pm 1.6\%$, respectively) comparing with the III group ($16.0\% \pm 0.6\%$). Headache as a manifestation of intoxication syndrome in the fever background was noted in $22.5\% \pm 1.8\%$ and $23.2\% \pm 1.7\%$ in the I and II groups, respectively, whereas in the III group significantly more often (in $49.5\% \pm 5.9\%$, $p < 0.05$). Younger patients were more likely to have an intoxication syndrome than older children ($9.1\% \pm 0.7\%$ and $2.8\% \pm 0.9\%$, respectively; $p < 0.05$).

The increase in body temperature to subfebrile digits was in $34.5\% \pm 4.7\%$ patients and to febrile digits was in $65.5\% \pm 8.6\%$ children of all age groups, and had no significant age difference ($p > 0.05$). Attention is drawn to the fact that in $4.5\% \pm$

0.9% of the II older group the signs of intoxication syndrome occurred on the normal temperature background.

The objective examination revealed that $89.0\% \pm 4.8\%$ of patients with CAUP had pallor of the skin and $31.3\% \pm 2.9\%$ had periorbital cyanosis; $30.8\% \pm 2.8\%$ had the symptoms of respiratory failure (RF) of the 1st degree. It should be noted that the skin pallor without RF signs was noted in $9.1\% \pm 0.9\%$ of the III group, in $19.0\% \pm 0.8\%$ of the II group and in $24.1\% \pm 0.9\%$ of the I group. The RF signs were noted in $8.1\% \pm 0.9\%$ of the I group, whereas in II and III groups $5.3\% \pm 0.7\%$ and $5.1\% \pm 0.6\%$, respectively.

The analysis of the cough character found the following: $88.7\% \pm 3.7\%$ dry cough, $11.3\% \pm 1.2\%$ moist cough in all the groups. In II and III groups, patients more often had dry cough than in the I group ($42.0\% \pm 7.7\%$, $33.0\% \pm 5.8\%$ and $28.1\% \pm 4.8\%$, respectively; $p < 0.05$).

The auscultative data analysis found that $86.2\% \pm 5.8\%$ had rales, of which $62.6\% \pm 5.8\%$ had wet rales and $26.2\% \pm 3.8\%$ had crepitant rales. Dry rales were heard in $17.8\% \pm 1.8\%$ of the younger age and $11.7\% \pm 1.5\%$ of the older age group. The dandruff, small and medium bubbly sounds were present in $82.6\% \pm 7.8\%$ and $62.6\% \pm 6.8\%$ of patients from respective groups, as well as their combination ($35.1\% \pm 3.8\%$ and $30.2\% \pm 3.6\%$, respectively). Auscultation patterns were not significantly associated with sex and PD level of patients ($p > 0.05$).

The analysis of the chest X-ray results showed that all the children had the inflammation process of a focal nature: $33.7\% \pm 5.7\%$ had right-sided, $47.0\% \pm 3.7\%$ had left-sided and $19.3\% \pm 1.8\%$ had two-sided inflammation. The probable differences in the radiographic data concerning the localization of the inflammatory process in patients with different levels of PD were not established ($p > 0.05$). With a deterioration of the general condition or absence of treatment dynamics, a repeated X-ray examination was performed in $17.5\% \pm 1.7\%$ of the I group, $9.7\% \pm 1.1\%$ of the II group and $11.6\% \pm 1.4\%$ of the III group.

The analysis of the results of the research of the cytokine profile (**Tab. 2**) established that in the acute period of the disease all children with CAUP, regardless of the level of PD, had a significant change in both pro- and anti-inflammatory cytokines in relation to their levels of control group. At the same time, the I and II groups had the rates of IL-1 β and IL-4 significantly higher

Table 2. Comparative characteristics of the levels of interleukins (IL-1 β , IL-4 and TNF- α) in blood serum in children with community-acquired uncomplicated pneumonia (CAUP).

Interleukins	Main groups							
	I group (OWT)		II group (BWD)		III group (APDI)		Control group	
	Research stages							
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
IL-1 β (pg/ml)	50.07 \pm 6.04	29.2 \pm 6.04 ^a	48.47 \pm 5.73 ^a	26.3 \pm 5.8 ^a	34.9 \pm 4.23 ^a	30.2 \pm 6.1 ^a	30.9 \pm 4.03	31.1 \pm 4.02
IL-4 (pg/ml)	25.24 \pm 2.05 ^a	29.2 \pm 2.05 ^a	31.69 \pm 2.34 ^b	34.5 \pm 2.34 ^b	24.24 \pm 2.64	26.22 \pm 5.1 ^a	19.24 \pm 2.04	19.3 \pm 2.05
TNF- α (pg/ml)	5.68 \pm 1.18 ^b	2.61 \pm 1.04 ^a	5.82 \pm 1.86 ^a	2.72 \pm 1.04 ^a	3.88 \pm 1.76 ^a	3.68 \pm 1.61 ^a	0.08 \pm 0.02	0.082 \pm 0.02

OWT: overweight; BWD: body weight deficiency; APDI: average physical development indices.

^ap < 0.05 in relation to the control group; ^bp < 0.05 in relation to the group with APDI.

in comparison with the III group. There was a significantly higher level of IL-4 in the II group in comparison with its indices of the III group, while there are no likely differences in this rate among children of the I and III groups.

The level of TNF- α in the acute period in children with BWD and OWT was significantly higher than with APDI. Increase in the concentration of IL-1 β and TNF- α in blood serum in children with BWD and OWT can lead to a low level of implementation of anti-infective protection and promote the spread of inflammatory process with CAUP [27].

After the treatment in all groups there was a decrease in the inflammatory process clinical manifestations, which was accompanied by a significant evidence decrease of the cytokine imbalance. Thus, at the second stage, on the treatment background, the probable decrease in the levels of IL-1 β and TNF- α is noted in all groups in comparison with the acute period indices. At the same time, the level of TNF- α after treatment is much higher in comparison with the control group. It is noteworthy that in the children with APDI on the treatment background the decrease in the level of TNF- α has no probable difference in comparison with the acute period, which may be due to its slight increase in the acute period in comparison with the levels in the I and II groups.

The level of IL-4 on the treatment background is likely to increase in children with CAUP, regardless of the PD level, remaining higher before recovery in comparison with control group. The children with BWD had IL-4 level significantly higher than those with APDI had, both during acute period and in the period of clinical improvement (at the 2nd stage), and it was significantly different from those of the control group.

The results of combined serological and bacteriological examination (sputum taken before

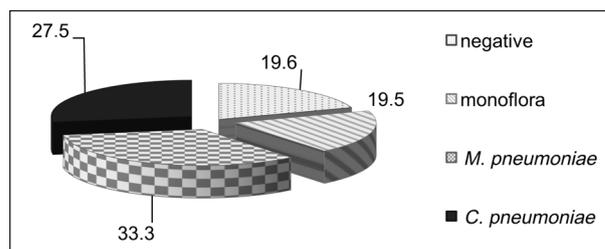


Figure 1. Results of combined serological and bacteriological examinations of the patients with community-acquired uncomplicated pneumonia (CAUP) (%).

the start of antibiotic therapy, on 2.2 \pm 0.8 day) allow to identify combinations of pneumotropic pathogens in children with CAUP (Fig. 1) and different levels of PD (Tab. 3).

The analysis of serological data (65.2% \pm 6.9%) showed a positive result concerning antibodies to causative agents of pneumonia (*M. pneumoniae* and *C. pneumoniae*). Monoflora was established in 19.5% \pm 3.9% with prevalence of positive serological markers of mycoplasma and chlamydial infections (33.3% \pm 2.9% and 27.5% \pm 3.2% of patients, respectively). 30.6% \pm 3.9% from the total number of examined patients had a combination of markers of pneumotropic infection.

The result shows that 5 (25% \pm 8.6%) of the control group had IgG to *C. pneumoniae* without increasing their concentration in paired serum.

It was established that 4.6 \pm 0.4% of the II group with positive result of serological markers of *S. pneumoniae* had IL-1 β and TNF- α levels remaining unchanged after treatment: 48.2 \pm 2.9 pg/ml and 6.68 \pm 0.48 pg/ml, respectively, at the first stage, and 48.0 \pm 2.8 pg/ml and 6.61 \pm 0.44 pg/ml, respectively, at the second stage of the study, that is, after treatment.

The result shows that 11.0% \pm 3.8% of the I group had the combination of causative agents of *M. pneumoniae* and *C. pneumoniae*; the markers of

Table 3. Results of serological and bacteriological examinations of the patients with community-acquired uncomplicated pneumonia (CAUP) and different levels of physical development (PD), n = 65.

Pathogen		Combined examination (serological and bacteriological), n = 37			Serological examination, n = 28			Total
		I group (OWT), n = 12	II group (BWD), n = 12	III group (APDI), n = 13	I group (OWT), n = 9	II group (BWD), n = 9	III group (APDI), n = 10	
Monoflora	<i>S. pneumoniae</i>	1	3	0	1	0	0	5
	<i>H. influenzae</i>	1	0	0	1	0	1	3
	<i>M. pneumoniae</i>	1	0	0	0	2	0	3
	<i>C. pneumoniae</i>	0	1	0	3	0	0	4
	Total	7			8			15
Combined pathogens	<i>M. pneumoniae</i> , <i>C. pneumoniae</i>	7	2	2	5	6	2	24
	<i>M. pneumoniae</i> , <i>C. pneumoniae</i> , <i>S. pneumoniae</i>	1	0	1	0	0	0	2
	<i>M. pneumoniae</i> , <i>C. pneumoniae</i> , <i>H. influenzae</i>	1	1	0	0	0	0	2
	<i>M. pneumoniae</i> , <i>H. influenzae</i>	1	0	1	0	0	0	2
	<i>M. pneumoniae</i> , <i>C. pneumoniae</i> , <i>S. pneumoniae</i> , <i>H. influenzae</i>	1	0	1	0	0	0	2
	<i>S. pneumoniae</i> , <i>C. pneumoniae</i>	0	1	0	0	0	0	1
	<i>S. pneumoniae</i> , <i>M. pneumoniae</i>	1	0	0	0	0	0	1
	<i>H. influenzae</i> , <i>C. pneumoniae</i>	0	1	0	0	0	0	1
	<i>M. catarrhalis</i> , <i>M. pneumoniae</i>	0	0	1	0	0	0	1
	<i>S. pyogenus</i> , <i>M. pneumoniae</i> , <i>C. pneumoniae</i>	0	1	0	0	0	0	1
Total	24			13			37	
Positive results		31			21			52
Negative results		6			7			13
Total together		37			28			65

OWT: overweight; BWD: body weight deficiency; APDI: average physical development indices.

IL-1 β and TNF- α in the acute period were 50.0 ± 3.8 pg/ml and 6.6 ± 0.8 pg/ml, respectively, while in the second examination 38.0 ± 4.8 pg/ml and 4.60 ± 0.44 pg/ml, respectively.

It was discovered that $8.0\% \pm 2.7\%$ of the I group, where causative agents of CAUP were combined infectious agents *M. pneumoniae* and *C. pneumoniae* and an increase in IL-4 (53.01 ± 1.7 pg/ml) was noted, there were manifestations of atopic dermatitis and a longer course of CAUP. In our opinion, this is due to the influence of IL-4, which stimulates the synthesis of IgE and IgG and promotes the formation of local tissue eosinophilia, weakening the local inflammatory response [11].

This prevents the effective destruction of infection in children with a history of allergies.

Discussion

Pneumonia is a disease associated to the individual, the infectious agent and the healthcare services [2, 19]. It is well known that about 200 individual cytokines with similar functional and biochemical characteristics are the participants in any inflammation, including pneumonia. Inflammation biomarkers, as cytokines, may be useful in determining the magnitude of the inflammatory response and lung injury in children

with pneumonia [9, 12, 13]. However, the studies that tried to associate clinical severity of respiratory diseases in children patients with serum levels of cytokines had controversial results [12, 28].

Pro- (IL-1 β and TNF- α) and anti-inflammatory (IL-4) interleukins take part in the activation of inflammation in course of CAUP in children and have certain features in patients with varying level of PD. The analysis of the possible relationship of the level of PD in children with CAUP with cytokine profile revealed that the children with OWT and BWD had more pronounced imbalance of pro- and anti-inflammatory cytokines, as opposed to the children with an average level of PD, which is reflected in the course of the disease. In a positive dynamics of the disease and a decrease in clinical manifestations of CAUP, the patients had a tendency towards normalization of markers of cytokine status, but their complete normalization did not occur. A study by Antunes et al. reported high levels of IL-6, TNF- α , IL-10 and IL-1 β detected in the majority of 24 patients with CAP at admission, but the cytokines levels decreased significantly in the end of hospitalization, as in our findings [29].

The PD level of the patients with CAUP was likely related to the levels of TNF- α and IL-1 β . We can assume that the interrelation between alveolar macrophages (possibly the recruited ones) in the lungs of children with OWT and BWD and the cytokines production affects both the reduction of phagocytic activity and the mechanisms of the first stage of protection from pathogenic microorganisms in the inflammatory site [30]. However, the children with OWT had a more significant positive dynamics in the level of anti-inflammatory cytokine (IL-4) in blood serum on the background of treatment, that is, at the 2nd stage of research.

Conclusion

The children with CAUP in the acute period had a probable increase in the levels of IL-1 β and TNF- α and IL-4 in blood serum (in relation to their rates in control group), which on the background of treatment probably decrease, not reaching the level of healthy children.

The children with OWT or BWD with CAUP had an imbalance mechanism violation between pro- and anti-inflammatory cytokines in the form of hyperproduction of IL-1 β and TNF- α and decrease in the production of the level of IL-4, which

promote more prolonged clinical manifestation in comparison with APDI patients.

The most significant symptoms of intoxication were observed in children with BWD that had positive serological markers of *S. pneumoniae*, which was accompanied by an increase in the level of IL-1 β and TNF- α , which did not have a positive dynamics during treatment.

Thus, the obtained data testify the possibility of considering the ratio of pro- and anti-inflammatory cytokines in the course of inflammatory process in children with CAUP with different PD as accessory factors to forecast the course of the disease.

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Declaration of interest

The Authors have no conflicts of interest to disclose.

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