УДК 616.24-002-053.2:577.175.7

PROGNOSTIC VALUE OF IL-6 AND IL-8 FOR RISK STRATIFICATION IN COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN

Natalya Anatolyevna Belykh *– MD, PhD, Head of the Department of Faculty and Outpatient Pediatrics, Ryazan State Medical University, Ryazan, Russian Federation.*

Natalya Aleksandrovna Anikeeva *– MD, PhD, Associate Professor, Department of Faculty and Outpatient Pediatrics, Ryazan State Medical University, Ryazan, Russian Federation.*

Anastasia Yuryevna Zhuleva *– Assistant Professor, Department of Faculty and Outpatient Pediatrics, Ryazan State Medical University, Ryazan, Russian Federation.*

Sultanova Nafisa Sabirovna S*.-DSc, Associate Professor, Department of Propaedeutic children diseases, Tashkent state medical University, Tashkent Uzbekistan*

Avezova Guloyim Sattarovna *- Associate Professor, Department of Propaedeutic children diseases, Tashkent state medical University, Tashkent Uzbekistan*

Abstract*. The search for prognostic biomarkers for the severity of community-acquired pneumonia (CAP) in children is of significant clinical interest.* ***The aim*** *of the study was to evaluate the serum interleukin-6 (IL-6) and interleukin-8 (IL-8) concentration in children with CAP depending on the severity of the disease.* ***Patients and methods.*** *The serum IL-6, IL-8 and procalcitonin (PCT) and C-reactive protein (CRP) concentrations were determined by ELISA upon admission in 37 hospitalized children (13 with severe CAP and 24 with non-severe CAP).* ***Results****. The serum IL-6 and IL-8 concentrations were significantly higher in the severe CAP group, compared of the non-severe CAP group by 2.2 and 3.6 times, respectively. Direct correlations of moderate strength were revealed between the severity of the disease and the serum IL-6 (r=0.68) concentration and IL-8 (r=0.57) respectively (p<0.0001). The serum IL-6 and IL-8 concentration correlated with each other (r=0.49, p=0.002) and with other inflammatory markers: serum IL-6 concentration with leukocytosis (r=0.42), neutrophilia (r=0.49), and PCT (r=0.53); serum IL-8 concentration – with PCT (r=0.49) and CRP (r=0.35).* ***Conclusion****. These data confirm the involvement of IL-6 and IL-8 in the pathogenesis of the systemic inflammatory response in CAP. Serum cytokines concentration are promising prognostic biomarkers for risk stratification of severe community-acquired pneumonia in children.*

Keywords*: community-acquired pneumonia, biomarkers, children, interleukin-6, interleukin-8.*

Аннотация*. Поиск прогностических биомаркеров тяжести внебольничной пневмонии (ВП) у детей представляет значительный клинический интерес.* ***Цель:*** *оценка уровня интерлейкина-6 (IL-6) и интерлейкина-8 (IL-8) в сыворотке крови у детей с ВП в зависимости от тяжести течения заболевания.* ***Материалы и методы.*** *У 37 госпитализированных детей (13 — с тяжелой ВП и 24 — с нетяжелой ВП) при поступлении определяли сывороточные концентрации IL-6, IL-8 и прокальцитонина (PCT) и С-реактивного белка (СРБ) методом ИФА.* ***Результаты****. Уровни IL-6 и IL-8 были достоверно выше в группе тяжелой ВП, превышая показатели группы пациентов с нетяжелой ВП в 2,2 и 3,6 раза соответственно. Выявлены прямые корреляционные связи средней интенсивности между тяжестью заболевания и уровнем IL-6 (r=0,68, p<0,0001) и IL-8 (r=0,57, p<0,0001). Концентрация IL-6 и IL-8 коррелировали друг с другом (r=0,49,*

p=0,002), а также с другими маркерами воспаления: уровень IL-6 — с лейкоцитозом (r=0,42), нейтрофилезом (r=0,49) и уровнем PCT (r=0,53); IL-8 — с PCT (r=0,49) и СРБ (r=0,35). ***Выводы****: Полученные данные подтверждают вовлеченность IL-6 и IL-8 в патогенез системного воспалительного ответа при ВП. Уровни этих цитокинов в сыворотке крови являются перспективными прогностическими биомаркерами для стратификации риска тяжелого течения внебольничной пневмонии у детей.*

Ключевые слова: *внебольничная пневмония, биомаркеры, дети, интерлейкин-6, интерлейкин-8.*

Rezyume. *Bolalarda shifoxonadan tashqari pnevmoniya (ShTP) og'irligini bashoratlovchi biomarkerlarni izlash muhim klinik ahamiyatga ega.****Maqsad:*** *shifoxonadan tashqari pnevmoniya bilan kasallangan bolalarda kasallik kechishining og'irligiga qarab qon zardobida interleykin-6 (IL-6) va interleykin-8 (IL-8) darajasini baholash.****Materiallar va usullar.*** *Shifoxonaga yotqizilgan 37 nafar bolada (13 nafari — og'ir ShTP, 24 nafari — og'ir bo'lmagan ShTP) qabul paytida qon zardobida IL-6, IL-8, prokaltsitonin (PCT) va C-reaktiv oqsil (CRO) miqdori immunoferment tahlil (IFT) usuli yordamida aniqlandi.****Natijalar.*** *Og'ir kechuvchi ShTP guruhi bolalarida IL-6 va IL-8 darajalari og'ir bo'lmagan ShTP guruhi ko'rsatkichlariga nisbatan mos ravishda 2,2 va 3,6 baravar yuqori ekanligi aniqlandi. Kasallik og'irligi bilan IL-6 (r = 0,68; p < 0,0001) va IL-8 (r = 0,57; p < 0,0001) darajalari o'rtasida o'rtacha kuchdagi to'g'ridan-to'g'ri korrelyatsion bog'liqliklar aniqlangan. IL-6 va IL-8 konsentratsiyalari o'zaro ham korrelyatsiyaga ega bo'lib (r = 0,49; p = 0,002), shuningdek yallig'lanishning boshqa markerlari bilan ham bog'liqlik ko'rsatdi: — IL-6 darajasi leykotsitoz (r = 0,42), neytrofilyoz (r = 0,49) va PCT darajasi (r = 0,53) bilan; — IL-8 darajasi PCT (r = 0,49) va CRO (r = 0,35) bilan korrelyatsiya qilgan.****Xulosalar.*** *Olingan natijalar shifoxonadan tashqari pnevmoniyada tizimli yallig'lanish javobi patogenezida IL-6 va IL-8 ning ishtirokini tasdiqlaydi. Ushbu sitokinlarning qon zardobidagi darajalari bolalarda shifoxonadan tashqari pnevmoniyaning og'ir kechish xavfini stratifikatsiya qilish uchun istiqbolli prognostik biomarkerlar hisoblanadi.*

Kalit so'zlar: *shifoxonadan tashqari pnevmoniya, biomarkerlar, bolalar, interleykin-6, interleykin-8.*

Relevance. In modern pediatric practice, the incidence of community-acquired pneumonia (CAP) is associated with increased mortality, a high rate of hospitalization, prolonged hospital stay, and frequent complications [3]. Early diagnosis of CAP can facilitate sound clinical decision-making and improve prognosis. Biomarkers such as white blood cell count, procalcitonin (PCT), and C-reactive protein (CRP) levels in serum are used in clinical practice to laboratory assess the severity of the disease and predict its course [4]. However, the use of traditional biomarkers has limited applicability for the diagnosis and prognosis of CAP in children. The search for informative indicators that, in addition to clinical assessment, can significantly improve the diagnosis and management of children with CAP and reduce adverse outcomes associated with this disease is urgent. Cytokines, which regulate the immune response and play a significant role in the pathogenesis of inflammation in CAP in children, may serve as such markers [1]. Cytokines are a vital link between various body systems (the immune, endocrine, nervous, and hematopoietic) and mediate their interactions in the development of inflammatory and immune responses. The leading mediators of infectious inflammation are interleukin 6 (IL-6) and interleukin 8 (IL-8) [5].

Interleukin-6 plays a key role in the regulation of both immune and inflammatory responses [7, 9]. During infection and tissue damage, IL-6 responds more quickly than leukocytes and CRP [6]. Some authors propose using this marker to predict the course of mycoplasma and adenoviral pneumonia in pediatric patients [8, 10]. Some studies have shown elevated IL-6 levels in severe pneumonia in children [1].

Interleukin -8 actively participates in the development of the inflammatory process.

Possessing high chemoattractant activity, it induces leukocyte chemotaxis followed by neutrophil degranulation, causes histamine release by mast cells, and stimulates angiogenesis [5]. Attempts have been made to evaluate changes in serum IL-8 concentration in children with CAP [2]. However, data confirming the value of determining IL-6 and IL-8 levels for the diagnosis, assessment of severity, and prognosis of CAP in children are insufficient.

The aim of the study was to evaluate serum IL-6 and IL-8 concentration in children with CAP, considering the severity of the disease, and to analyze the relationships between these parameters and the severity of the pathology and biomarkers of systemic inflammation.

Materials and methods. A total of 37 children with a diagnosis of CAP verified according to clinical guidelines were observed. The study complies with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (2000) and was approved by the university's Ethics Committee (Protocol No. 5 of the Ethics Committee meeting dated November 7, 2023). Informed voluntary consent was obtained from all participants. The authors express their gratitude to the university administration and the staff of the Central Research Laboratory for their assistance in conducting the study.

Inclusion criteria: age from 1 month up to 17 years 11 months, diagnosis of CAP verified according to clinical guidelines, no antibacterial therapy at the outpatient stage.

Exclusion criteria: age under 1 month and/or over 17 years 11 months, antibacterial therapy at the outpatient stage, presence of diseases of the bronchopulmonary system and concomitant pathology affecting respiratory function, presence of other inflammatory diseases.

Clinical and laboratory examination of patients and diagnosis of CAP were carried out in accordance with adaptations with current clinical guidelines [4]. The average age of the patients was 10.8±5.5 years; there were 17 girls (45.9%) and 20 boys (54.1%) with CAP. The patients were divided into 2 groups: 1st group – patients with severe CAP (n=13) and 2nd group – patients with non-severe CAP (n=24). The vaccination history of patients in both groups did not differ significantly: 6 (46.2%) children in the 1st group and 10 (41.7%) children – in the 2nd group were vaccinated against pneumococcal infection, 5 (38.5%) and 6 (25.0%), respectively, against H. influenzae infection, and 1 (7.7%) and 3 (12.5%) against influenza.

All patients had their blood drawn upon hospitalization, and serum IL-6, IL-8 and PCT concentrations were determined by heterogeneous enzyme-linked immunosorbent assay (ELISA) using commercial Human IL-6 ELISA Kit, Human IL-8, and Human PCT ELISA Kit (Wuhan Fine Biotech Co., Ltd. (FineTest), China). Light absorbance was measured at 450 nm on a Stat Fax 2100 enzyme-linked immunosorbent assay plate reader (Awareness Technology, USA). Serum interleukin concentrations were expressed as pg/ml, and PCT concentrations were expressed as ng/ml. Serum CRP concentration were determined by immunoturbidimetry using reagent kits from DiaSys, Germany. Measurements were performed on a Sapphire 400 biochemical analyzer. Premium, Japan. Serum CRP concentrations were expressed as mg/ml. Leukocyte and neutrophil counts in patients' blood were assessed using a complete blood count.

Statistical processing of the obtained data was performed using the GraphPad Prism8 software package. The Mann-Whitney test was used to compare independent samples. Differences in the compared parameters were considered significant at $p<0.05$. To identify correlations between parameters, Spearman's rank correlation coefficients (r) were calculated with a significance level (p).

Results and Discussion. Children with CAP, regardless of the severity of the disease, were admitted to hospital on days 6-7 from the onset of the first symptoms of the disease. In the clinic of the disease in patients with CAP in both groups, symptoms of respiratory tract damage and intoxication predominated. However, in children with severe CAP (Group 1st), compared with patients in Group 2nd (non-severe CAP), symptoms of intoxication such as weakness (92.3 vs. 68.2%, $p=0.038$), tachycardia (89.5 vs. 37.5%, $p<0.0001$), and decreased appetite (76.9 vs.

46.6%, p=0.006) were significantly more common. Manifestations of respiratory failure were also observed in the form of decreased saturation <95% (53.8 *vs*. 4.2%, p<0.0001), mixed dyspnea with the involvement of accessory muscles in the act of breathing (76.9 *vs*. 16.7%, p<0.0001) and tachypnea (68.4 *vs*. 25.0%, p<0.0001).

When assessing the levels of the studied serum interleukins (IL-6 and IL-8) concentration in patients with CAP at the time of hospitalization, their dependence on the nature of this disease was revealed - serum both interleukins concentrations were significantly higher in children with severe CAP compared to patients with non-severe CAP. Thus, upon admission to hospital, the serum IL-6 concentration in children in group 1st was in 2.2 times higher than in children in group 2nd (32.8 [24.1; 41.7] *vs*. 15.0 [8.5; 20.0] pg/ml), p<0.0001 (Fig. 1). Moreover, the serum IL-8 concentration in 1st group was in 3.6 times higher than in 2nd group (80.9 [45.6; 92.7] *vs*. 22.3 [11.0; 33.0] pg/ml), p<0.0001 (Fig. 2).

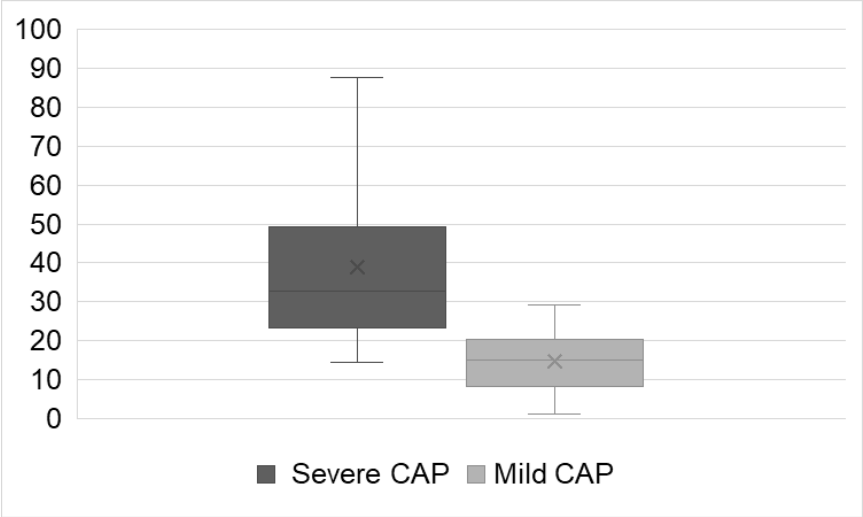

Figure 1. Serum IL-6 concentration in children with CAP. Key: Y-axis – serum IL-6 concentration (pg/ml).

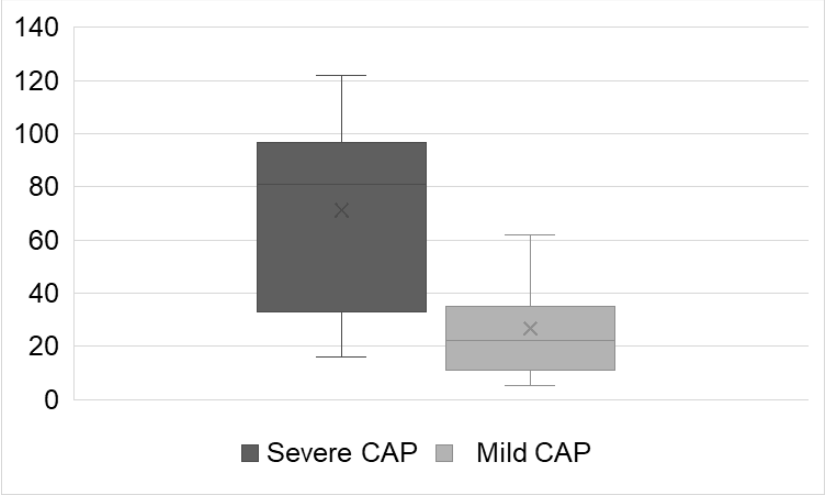

Figure 2. Serum IL-8 concentration in children with CAP. Key: Y-axis – serum IL-8 concentration (pg/ml).

Spearman's rank correlation analysis revealed direct moderate correlations between the severity of CAP and serum levels of IL-6 (r=0.68, p<0.0001) and IL-8 (r=0.57, p<0.0001).

Analysis of laboratory criteria for systemic inflammation revealed that in children with severe CAP, upon admission to hospital, most of the indicators were significantly higher than normal, while in children with non-severe CAP they were significantly lower, and many of them

were within the reference values. Thus, in children in 1st group upon admission, the number of leukocytes reached 15.1 [9.4; 18.4] * 10^9/l, which was almost in 2.5 times higher than in the 2nd group - 6.3 [4.6; 7.8] x 10^9/l (p=0.002). The same pattern was noted in terms of the absolute number of neutrophils: in patients in 1st group it was in 2 times higher than in 2nd group (9.1 [6.6; 13.3] x10^9/l and 4.3 [3.2; 6.4]x10^9/l, respectively, p=0.004). The serum PCT concentration in 1st group was 1.02 [0.78; 4.70] ng/ml, more than in 3 times higher than in 2nd group - 0.31 [0.26; 0.47] ng/ml (p<0.0001). The serum CRP concentration was also in 1.7 times higher in 1st group compared of 2nd group 2 (48 [24; 96] *vs*. 28 [24; 48] mg/l), but this difference was not statistically significant (p=0.904). These data indicated the development of a systemic inflammatory response in children with severe CAP, which was confirmed by the identified correlations between the severity of the pathology and leukocytosis/neutrophilia (r=0.65/0.63, p<0.001) and serum PCT concentration (r=0.75, p<0.001).

Furthermore, Spearman's rank correlation analysis revealed significant correlations between the studied serum interleukins (IL-6 and IL-8) concentration both among themselves (r=0.49, p=0.002) and with other studied biomarkers of systemic inflammation. In particular, serum IL-6 concentration correlated with leukocytosis (r=0.42, p=0.009), absolute neutrophil count (r=49, p=0.002), and serum PCT concentration (r=0.53, p<0.001), while serum IL-8 concentration correlated with PCT (r=0.49, p=0.002) and CRP (r=0.35, p<0.05). These data are consistent with studies by a number of authors who have demonstrated the indicative value of serum IL-6 and IL-8 concentration in reflecting the severity of CAP in children and adults [2,8,9].

The identified relationships indicate the involvement of these interleukins in the development of CAP against the background of a systemic inflammatory response associated with this pathology. Short-term IL-6 expression is believed to contribute to host cell protection from infection and tissue damage by stimulating the acute-phase immune response and hematopoiesis, and its synthesis ceases when homeostasis is restored [7]. Our data indicate that in children with severe CAP, serum IL-6 concentration was significantly elevated, as was the serum IL-8 concentration, which, as a potent chemoattractant, stimulates the phagocytic activity of immunocompetent cells [5]. The identified correlation between these interleukins clearly indicates their synergistic effect in the development of CAP.

Conclusion. The obtained results and their analysis indicate that the development of severe CAP in children is associated with increased serum proinflammatory cytokines such as IL-6 and IL-8concentration, as well as the studied laboratory parameters of the systemic inflammatory response, including leukocytosis, serum PCT and CRP concentrations. The identified correlations between the levels of the studied interleukins and the severity of CAP in children and laboratory indicators of the systemic inflammatory response indicate their pathogenetic involvement in the development of an unfavorable course of this pathology.

The identified changes in serum IL-6 and IL-8 concentration in children with CAP allow these indicators to be considered promising biomarkers for assessing the severity of CAP in pediatric patients. The data obtained can be further used to develop personalized diagnostic algorithms and predict the course of CAP in children.

References

1. Izyurova N.V., Savochkina A.Yu., Uzunova A.N., Nokhrin D.Yu. Cytokine profile in community-acquired pneumonia in children // Medical Immunology. - 2022. - Vol. 24, No. 5. - Pp. 943-954. DOI: 10.15789/1563-0625-CPI-2538

Iziurova N.V., Savochkina A.Yu., Uzunova A.N., Nokhrin D.Yu. Cytokine profile in community-acquired pneumonia in children, Medical Immunology (Russia). Meditsinskaya Immunologiya, 2022, Vol. 24, No. 5, pp. 943-954. DOI: 10.15789/1563-0625-CPI-2538

2. Malanicheva T.G., Mozhgina S.S., Agafonova E.V. Local cytokine profile and cytological status in children with community-acquired pneumonia occurring against the background of

reduced resistance of the body // Russian Bulletin of Perinatology and Pediatrics. – 2017. – Vol. 62, No. 5. – P. 139-143. DOI: 10.21508/1027–4065–2017–62–5–139–143

Malanicheva T.G., Mozhgina S.S., Agafonova E.V. Local cytokine profile and cytological status in children with community-acquired pneumonia arising on the background of the reduced resistance of the organism. Russian Bulletin of Perinatology and Pediatrics, 2017, Vol.62, no. 5, pp. 139–143. (in Russ). DOI: 10.21508/1027–4065–2017–62–5–139–143

3. On the state of sanitary and epidemiological well-being of the population in the Russian Federation in 2023: State report. – Moscow: Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, 2024. – 364 p.

On the state of sanitary and epidemiological well-being of the population in the Russian Federation in 2023: State report. Moscow: Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, 2024, 364 p.

4. Pneumonia (community-acquired). Clinical guidelines – 2022-2023-2024 (January 18, 2022): Approved by the Ministry of Health of the Russian Federation / Union of Pediatricians of Russia. – Moscow, 2022.

Pneumonia (community-acquired). Clinical guidelines – 2022-2023-2024 (01/18/2022): Approved by the Ministry of Health of the Russian Federation / Union of Pediatricians of Russia. Moscow, 2022. http://disuria.ru/_ld/11/1132_kr22J12-J18MZ.pdf

5. Simbirtsev A.S. Cytokines in the pathogenesis and treatment of human diseases. St. Petersburg: Foliant, 2018, 512 p.

6. Karakioulaki M., Stolz D. Biomarkers in pneumonia – beyond procalcitonin. Int J Mol Sci. 2019, Vol. 20, no. 8, pp. 2004. DOI: 10.3390/ijms20082004

7. Tanaka T., Kishimoto T. The biology and medical implications of interleukin-6. Cancer Immunol Res., 2014, Vol. 2, no 4, pp. 288-294. DOI: 10.1158/2326-6066.CIR-14-0022

8. Wang H., Zhang Y., Zhao C., Yun Peng, Wenqi Song, Weihan Xu et al. Serum IL-17A and IL-6 in pediatric Mycoplasma pneumoniae pneumonia: implications for different endotypes. Emerg Microbes Infect., 2024, Vol. 13, no 1, pp. 2324078. DOI: 10.1080/22221751.2024.2324078

9. Weidhase L., Wellhöfer D., Schulze G., Kaiser T., Drogies T., Wurst U., Petros S. Is interleukin-6 a better predictor of successful antibiotic therapy than procalcitonin and C-reactive protein? A single center study in critically ill adults. BMC Infect Dis., 2019, Vol. 19, pp.150. DOI: 10.1186/s12879-019-3800-2

10. Yao G., Ma C., Liu J., Zexuan Sun, Botao Wei. Interleukin-6 serum levels are independently associated with severeadenovirus pneumonia in children: a cross-sectional study. Transl Pediatr., 2022, Vol. 11, No. 12, pp. 1962-1971. DOI: 10.21037/tp-22-585.